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BETA AND DELTA CELLS OF THE HUMAN ADENOHYPOPHYSIS: THEIR RESPONSE TO ADRENOCORTICAL DISORDERS*

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ABSTRACT

Five types of cells can be distinguished in the human adenohypophysis by the iron-PAS technique; alpha cells (acidophils), beta and delta cells (two types of basophils), gamma cells (a heterogenous group of lightly PAS-positive cells), and chromophobes (small nongranular cells). Cell counts were performed on the hypophysis in 7 cases of Addison's disease, 3 cases of Cushing's syndrome, and 14 patients who had received large doses of glucocorticoids. The results were compared to the counts in a control group of 71 subjects without endocrine disease. In adrenal insufficiency the proportion of Crooke-Russell gamma cells was increased, and that of alpha and beta cells was decreased. Conversely, in states of prolonged glucocorticoid excess the proportion of gamma cells was decreased, and that of alpha cells was increased. Many beta cells were hyalinized, although their total percentage remained unchanged. With either adrenal insufficiency or excess of glucocorticoids, delta cells often persisted at a higher level than was found in chronic nonendocrine illnesses. The following responses were shown by beta cells but not by delta cells: beta cells were consistently reduced in Addison's disease; they were the site of Crooke's hyaline change in Cushing's syndrome; and they made up all the basophil adenomas observed, with or without Cushing's syndrome. The difference in staining properties between beta and delta cells was associated with differences in the

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response to disturbances in adrenocortical function. It is suggested that both beta and gamma cells produce corticotropin. Gamma cells are actively secreting forms with little storage of granules; beta cells store enough granules to permit intense staining.

IN RECENT years many new stains have been applied to the anterior pituitary gland. This has led to considerable difficulty in the identification of corresponding cell types described by various investigators. By examining specific responses to disturbances in endocrine function, it should be possible to recognize and identify the same cell type with different staining techniques.

With the iron-PAS[1] technique, we found that 2 strongly PAS-positive cells, beta and delta, could be distinguished in the human adenohypophysis (1). Besides staining differently, these cells had a characteristic pattern of distribution in the gland. Another difference was that delta cells were not found in children or pregnant women, whereas beta cells were present in these subjects. Also, the proportion of delta cells, unlike that of beta cells, was significantly decreased in association with increasing duration of the final illness.

The purpose of the present work was to study the response of beta and delta cells to disturbances in adrenocortical function.

METHODS

Pituitary glands obtained from routine necropsy cases at the Toronto General Hospital were stained by the iron-PAS technique. When the history indicated adrenocortical disease or treatment with glucocorticoids or corticotropin (ACTH), the cases were selected for special study. Additional cases of interest were obtained from other hospitals.

The technical details of fixation, cutting and staining have been published (1). With this technique, at least 5 types of cell can be distinguished, *viz*, alpha, beta, gamma, delta and chromophobe. These cells are described in detail in our previous publication. The orange-staining PAS-negative alpha cells are classic acidophils. Beta cells are PAS-positive basophils with red granules; delta cells are another type of PAS-positive cells, with granules that stain blue-purple. Gamma cells are a heterogenous group of light-purple PAS-positive cells, which with other stains often appear as large chromophobes. True chromophobes are small cells devoid of granules.

Cell counts, according to the method previously described (1), were performed on all suitable glands. In our earlier investigation (1), it was noted that delta cells were decreased in nonendocrine illnesses of long duration. The relative proportions of the other cell types were not influenced significantly by the length of the terminal illness. Therefore, composite average of the 3 groups of patients from our previous study who died from nonendocrine diseases of various durations (sudden death, short illness, long illness) were established for alpha, beta and gamma cell and chromophobe counts. These composite averages were used as controls for the present investigation (Table 1). It was thought that both Addison's disease and Cushing's syndrome should be considered ill-

[1] Periodic acid-Schiff.

nesses of long duration, even if the terminations were acute. All the patients in our series who had been treated with adrenal steroids had been ill for longer than two weeks. The delta cell counts from the present study were, therefore, compared to those in the long-illness group from our previous report.

RESULTS

A. *Adrenocortical insufficiency* (Tables 1 and 2)

The pituitaries from 12 patients with Addison's disease who had died of adrenal insufficiency were examined. Seven of these glands were suitable for counting.

The most notable feature was the highly significant reduction in beta cells. The proportion of beta cells in any gland was no more than 3 per cent and in 2 glands there were no beta cells to be seen. *In contrast to the scarcity of beta cells, the percentage of delta cells was very high in 2 of these glands and moderate in 2 others.* It was low in the remaining 3 cases.

The alpha cells were small and poorly stained, and their proportion was reduced significantly. This reduction in beta and alpha cells resulted in the glands having a predominantly chromophobic appearance. The percentage of true chromophobes was significantly increased, and the gamma cell count was in the high range of normal. Most of the gamma cells were

TABLE 1. THE EFFECT OF ADRENAL DISORDERS ON THE PROPORTION OF PITUITARY CELL TYPES

Endocrine condition	No. of cases	Proportion of cell types				
		Alpha %	Beta %	Delta %	Gamma %	Chromophobe %
Nonendocrine control*	71	50.31±2.6†	13.01±1.4	a) 8.46±0.8 b) 4.56±0.7 c) 1.93±0.4	8.33±0.6	23.04±2.4
Adrenocortical insufficiency	7	29.18±4.1	1.21±0.5	4.42±2.0	16.70±4.1	48.47±2.7
Glucocorticoid therapy	14	60.12±2.1	12.30±1.6	3.27±0.6	4.48±0.4	20.28±2.7
Statistical Analysis: P Values (less than 0.05)						
Adrenocortical insufficiency : nonendocrine control		0.001	0.001	—	—	0.001
Glucocorticoid therapy : nonendocrine control		0.02	—	—	0.001	—
Glucocorticoid therapy : adrenocortical insufficiency		0.001	0.001	—	0.02	0.001

* The percentages of alpha, beta and gamma cells and chromophobes are the composite averages of data on the 3 groups of patients from our previous study who died of nonendocrine illnesses of various durations (1). These were used as controls for the present investigation. The percentages of delta cells are listed separately for the 3 groups: a) sudden death (less than 24 hours); b) short illness (1–14 days); c) long illness (more than 2 weeks). Only the long-illness group (c) was used as a control for the delta cells of the present series, since endocrine illnesses were considered to be of long duration.

† Standard error

hypertrophic. Many showed an accumulation of blue-purple granules near the nucleus, often associated with a prominent negative image of the Golgi apparatus (Fig. 1). Such cells were described by Crooke and Russell (2) and have since been referred to as Crooke-Russell cells. They are found occasionally in actively secreting glands not associated with adrenal deficiency, but are most numerous in pituitaries from patients with Addison's disease.

In 1 case of Addison's disease (A-282-47), untreated myxedema was also present at the time of death. The pituitary was composed primarily of large gamma cells, many of which contained vesicles commonly associated

TABLE 2. THE ADENOHYPOPHYSIS IN ADRENAL INSUFFICIENCY (ADDISON'S DISEASE)

Autopsy No.	Sex & age (yrs.) of patient	Endocrine condition* or treatment	Proportion of cell types				
			Alpha %	Beta %	Delta %	Gamma %	Chromophobe %
A.1918/41	M.20	Adrenal atrophy, 2 yrs.	30.8	3.0	11.4	5.0	50.2
A.72/43	M.39	Adrenal atrophy, 2 yrs.	32.2	1.5	9.9	12.4	44.2
P.34/57	M.—	Tuberculosis adrenal gland; no treatment.	43.6	0.0	3.7	10.2	42.7
P.41/57	M.—	Tuberculosis adrenal gland; no treatment.	22.6	0.0	0.0	21.2	56.0
A.299/37	M.51	Tuberculosis adrenal gland, 1 yr.	28.1	1.5	1.5	10.0	59.2
A.421/35	F.55	Tuberculosis adrenal gland; symptoms 4 mos.	37.0	2.5	0.5	21.1	39.0
A.282/47	F.37	Thyroidectomy 1926 led to myxedema. Addison's disease since 1937. No thyroid therapy for several years before death.	10.0	0.9	4.2	37.0	48.0
		Average (7 cases) ±Standard Error	29.18 ±4.1	1.21 ±0.5	4.42 ±2.0	16.70 ±4.1	48.47 ±2.7

* The cause of death in all these cases was a crisis of adrenocortical insufficiency.

NOTE: 1. There was a significant decrease in alpha and beta cells, and a significant increase in chromophobes, in comparison to the control group. In comparison to the glucocorticoid-treated group, these differences were even more pronounced, and in addition there were significantly more gamma cells in the adrenal insufficiency group.

2. Many of the gamma cells showed the characteristics of Crooke-Russell cells (2).

3. In the hypophysis of Patient A.282/47, in whom Addison's disease was associated with myxedema, there were both Crooke-Russell and vesiculated gamma cells.

with myxedema (3–5). Crooke-Russell gamma cells, characteristic of Addison's disease, were also present. With myxedema and adrenal insufficiency co-existing in this patient, the pituitary combined the pathologic features of both diseases.

B. *Adrenal glucocorticoid excess* (Tables 1 and 3)

Spontaneous adrenal oversecretion. The pituitaries from 3 cases of spontaneous Cushing's syndrome were studied. Two of them contained basophil adenomas large enough to compress the surrounding gland. The adenomas were excluded from the cell counts. In both cases the beta cells outside the tumor were present in normal proportions. About half of these

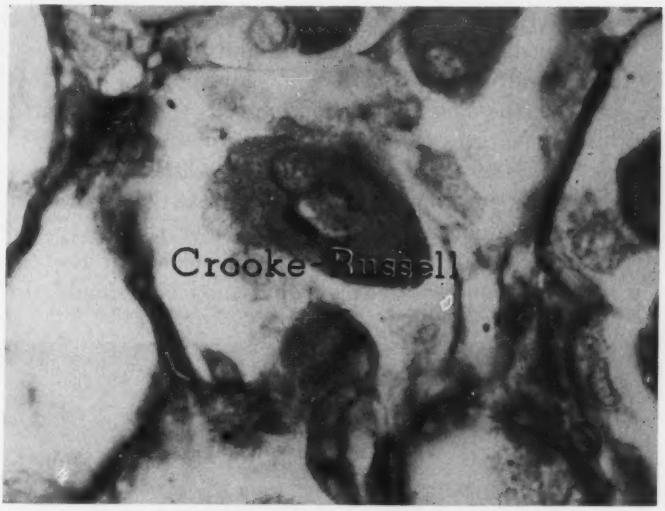

FIG. 1. Histologic section of the anterior pituitary in a patient with Addison's disease due to tuberculosis of the adrenal glands (A-299-37, male, aged 51), showing a typical Crooke-Russell gamma cell with a prominent negative image of the Golgi apparatus, surrounded by purple granules. Iron-PAS stain ×1350.

beta cells showed the hyaline cytoplasmic change[2] first described by Crooke in 1935 (6) (Fig. 2). The third pituitary did not contain an adenoma. Although the beta cells were few in number, half of them were classified as Crooke's hyaline cells. *The hyaline change was not observed in any delta cells.*

The adenomas associated with Cushing's syndrome were composed entirely of beta cells, none of which was hyalinized. Basophil adenomas are found rarely in routine pituitary surveys (7). Only 6 cases of basophil adenoma without Cushing's syndrome were observed over the past twenty-five years in routine autopsies at the Toronto General Hospital. These adenomas were all small, and when restained with the iron-PAS stain, were seen to be composed entirely of beta cells. *No tumor of delta cells was seen.* Delta cells were absent from the rim of normal gland tissue in the 2 pituitaries with adenomas associated with Cushing's syndrome. The lack of delta cells in these cases may be related to compression of the surrounding tissue by the tumor.

The association of hyalinized beta cells with glucocorticoid excess rather

[2] All cells which showed any degree of hyalinization were counted as Crooke's hyaline cells. The percentage is somewhat higher than is generally reported in the literature, since in many such studies only fully developed hyaline cells were counted.

than androgen excess was confirmed by the following observations: In 2 cases of adult virilism without evidence of glucocorticoid excess, and in 4 cases of virilizing adrenal hyperplasia in children,[3] no Crooke's hyaline cells were seen. On the other hand, hyaline cells were found in 3 cases of virilism with some features of Cushing's syndrome.

Therapeutic administration of adrenal glucocorticoids. The pituitary changes in Cushing's syndrome may be the cellular representation of overproduction of a certain type of corticotropin, or they may represent a regressive response to high levels of glucocorticoids in the circulation. Changes which are secondary to excess glucocorticoids should also be seen

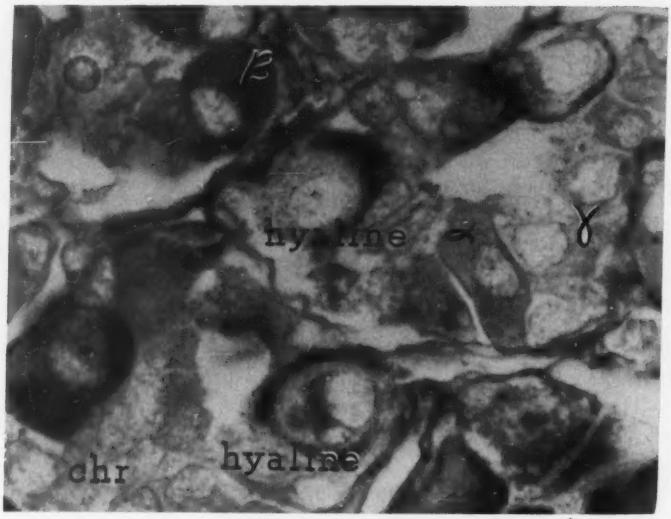

FIG. 2. Histologic section of the anterior pituitary in a patient with disseminated lupus erythematosus treated by large doses of glucocorticoid hormones (see Table 6) (A/361/55, male, aged 25), showing several typical Crooke's hyaline beta cells in various stages of hyalinization. Iron-PAS stain ×1350.

in the pituitaries of patients who had received exogenous glucocorticoids or ACTH. As an aid in distinguishing primary and secondary changes, the pituitaries from 14 patients who had been treated with cortisone or its analogues (*e.g.*, hydrocortisone) for periods ranging from three weeks to several years were counted. Six of these subjects had received some form

[3] Three of these glands were obtained through the courtesy of Dr. Ella Oppenheimer and Dr. Lawson Wilkins of the Johns Hopkins Hospital, Baltimore, Maryland. The fourth was obtained from the Hospital for Sick Children, through the courtesy of Dr. W. L. Donohue, Pathologist-in-Chief.

TABLE 3. THE ADENOHYPOPHYSIS IN ADRENAL GLUCOCORTICOID EXCESS

Autopsy No.	Sex & age (yrs.) of patient	Endocrine condition or treatment	Terminal illness*	Proportion of cell types					
				Alpha %	Beta		Delta %	Gamma %	Chromophobe %
					normal %	hyaline %			
a) *Spontaneous adrenal oversecretion* (Cushing's syndrome)									
A.445/56**	F. 70	Adrenal hyperplasia, 6 yrs.	Cerebral infarction	54.2	4.2	5.9	0	4.1	32.0
P.23/56**	F. 47	Adrenal hyperplasia, 1 yr.	Bronchopneumonia	51.8	7.0	7.1	0	8.3	25.6
A.369/58	M. 35	Adrenal hyperplasia, 5 yrs. Bilat. adrenalectomy 1 mo. before death. Treated with 75–150 mg. cortisone daily postop.	Pulmonary fibrosis; complications following gastrectomy	76.0	2.1	1.1	3.8	3.3	13.2
b) *Therapeutic administration of adrenal glucocorticoids*†									
A.18/57	M. 51	Cortisone, prednisone, ACTH	Polyarteritis; bacterial endocarditis	67.3	7.1	7.1	6.5	4.4	13.2
A.371/55	F. 27	Prednisone, ACTH	Thrombotic purpura	59.0	9.6	3.2	0	2.7	25.0
A.361/55	M. 25	Cortisone, prednisone, ACTH	Disseminated lupus	65.4	4.5	4.3	6.1	3.7	16.4
A.270/57	F. 52	Prednisone, ACTH, hydrocortisone	Pancytopenia	51.2	5.7	4.9	1.5	3.7	32.8
A.160/56	F. 29	Cortisone, ACTH	Disseminated lupus	54.3	0.9	2.2	0	3.1	40.5
A.135/55	M. 40	Cortisone, ACTH	Cirrhosis; ulcerative colitis	60.0	5.1	2.9	1.2	2.0	29.0
A.150/58	F. 16	Prednisone	Lymphatic leukemia	77.0	3.9	2.2	4.2	7.5	7.4
A.135/56	F. 34	Prednisone	Rheumatoid arthritis; amyloidosis	66.0	4.9	7.4	3.5	4.5	13.4
A.194/58	F. 36	Prednisone	Lymphatic leukemia	55.5	5.8	2.4	2.5	4.7	28.9
A.72/58	M. 61	Prednisone	Pancytopenia following chloramphenicol	58.0	5.1	5.6	2.5	4.9	25.0
A.495/56	M. 50	Prednisone	Malignant hypertension	61.0	14.0	5.8	3.6	7.5	7.3
A.59/56	M. 83	Prednisone	Multiple myeloma	45.4	17.4	7.4	3.6	5.3	20.0
A.177/56	M. 43	Cortisone	Leukemia	55.6	12.6	8.4	3.6	5.6	14.5
A.320/56	M. 31	Hydrocortisone, prednisone	Scleroderma, uremia	66.1	8.7	4.2	7.1	3.1	10.6
Average (14 cases)‡ ±Standard Error				60.12 ±2.1	7.52 ±1.2	4.86 ±0.6	3.27 ±0.6	4.48 ±0.4	20.28 ±2.7
Total beta cells				12.30±1.6					

* In all cases the duration of the terminal illness was longer than 2 weeks.

** In each of these 2 cases there was a beta-cell adenoma of sufficient size to compress the surrounding tissue. None of the cells in the tumor was hyalinized. Cell counts did not include the adenomas.

† The dosage varied, but was usually greater than the equivalent of 75 mg. of cortisone daily for longer than 2 months. Treatment was continued until the time of death.

‡ Average of cases with glucocorticoid treatment (group (b)). Because only 3 cases of spontaneous Cushing's syndrome were available, no statistical analysis was made in this group (a).

NOTE: 1. In comparison to the control group, there was a significant increase in alpha cells, and a significant decrease in gamma cells.
2. Beta cells were present in normal proportions, but almost half of them showed some degree of hyalinization.
3. In many patients with illnesses of long duration there was a high proportion of delta cells.

of corticotropin as well. The pituitary of 1 patient who had received ACTH only was examined, but the cells were not counted.

A hyaline change in the beta cells similar to that observed in Cushing's syndrome was seen consistently after prolonged glucocorticoid or ACTH therapy; about half the beta cells were affected. *No hyalinized delta cells were seen.*

This group was large enough to permit statistical analysis of the cell counts. The total proportion of beta cells (including both hyalinized and normal cells) did not differ from that observed in the control series, but was significantly higher than in the adrenal insufficiency group. The proportion of alpha cells was increased and that of gamma cells was decreased significantly, when compared to the normal or to the Addisonian group. In several instances the percentage of delta cells was high for patients with a chronic illness, but because of variation in the group, the increase in delta cells was not statistically significant.

There were not enough cases of spontaneous Cushing's syndrome to justify a separate statistical analysis. However, if the counts from the 3 cases of Cushing's syndrome were included with the counts from the glucocorticoid therapy group, the averages would not be altered appreciably.

DISCUSSION

Examination of the hypophysis in adrenocortical disorders may help to determine which variety of anterior pituitary cell secretes ACTH. Our studies confirmed previous observations that the pituitary in cases of adrenal insufficiency contains numerous large *gamma* cells, many of which are of the Crooke-Russell type (2, 8). Gamma cells ("amphophils") present some difficulty in classification. Usually they stain lightly basophilic and PAS-positive; occasionally they have acidophilic properties (8). Although gamma cells may stain like either beta or delta cells, according to the stain used, gamma cells may be recognized by the fact that their staining reaction is less intense. With the iron-PAS stain, as well as with PAS-methyl blue stain (9), gamma cells stain purple like delta cells. In a previous paper (9) these gamma cells were called PAS-purple cells, and were considered to be modified delta cells. Since then, we have come to regard gamma cells as a heterogenous group of PAS-positive cells, some of which may arise from beta cells. Indeed, with the aldehyde-fuchsin technique, the Crooke-Russell gamma cells of Addison's disease exhibit a beta-type color reaction (10, 11). Much of the confusion in the nomenclature of pituitary basophils would be eliminated if the term *delta* cell were reserved for the coarsely granulated, darkly staining PAS-positive cells which occur singly in all regions of the adenohypophysis, and the term *beta* cell were kept for the classic heavily-granulated polyhedral basophils which occur in clumps and are concentrated in the antero-medial portion of the gland.

There is general agreement that pituitaries containing many gamma cells are actively secreting glands (2, 8, 10–13). In untreated Addison's disease there is an increased blood level of ACTH which probably represents increased production. Therefore, it seems likely that in adrenal insufficiency the gamma cells, especially those of the Crooke-Russell variety,

are secreting ACTH. The other changes in the proportion of pituitary cell types in Addison's disease, namely, the decrease in beta and alpha cells and the increase in chromophobes, may be nonspecific responses to the low levels of glucocorticoids. On the other hand, there may be a transformation of one or both varieties of the chromophils to actively secreting gamma cells. The decrease in alpha cells probably bears no direct relation to ACTH production, since alpha cells are also decreased in hypothyroidism (5, 8, 10). The occasional occurrence of microsplanchnia in Addison's disease suggests that the decrease in alpha cells is sometimes associated with decreased production of somatotropin (2).

In contrast to the cases of adrenal insufficiency with excessive secretion of ACTH are the cases treated with such large amounts of exogenous glucocorticoids that ACTH output is reduced. The pituitaries in patients treated with adrenal steroids show a reduction in the proportion of gamma cells and of chromophobes, an increase in the proportion of alpha cells, and a hyaline change in the beta cells.

The decrease in gamma cells may be related to decreased production of ACTH. The hyaline change in the beta cells is a specific cellular lesion that is apparently brought about by a prolonged excess of glucocorticoids (8, 13–17). That the beta cell is singled out for this alteration suggests that it, too, is linked with ACTH production.

In spontaneous Cushing's syndrome most of the changes in the pituitary are similar to those found in association with glucocorticoid therapy and are, therefore, probably secondary to glucocorticoid excess. There is one pituitary abnormality sometimes found in Cushing's syndrome which has not been produced by even prolonged administration of cortisone or its analogues—namely, adenoma. In the 2 cases in our series the tumors were composed of beta cells. The "basophil" and "chromophobe" adenomas reported with Cushing's syndrome (14, 18–20) are probably composed of beta and gamma cells respectively. Although the etiology of Cushing's syndrome is obscure, when adenomas are present it is likely that these tumors are secreting some type of corticotropic hormone. The contrast between the pituitaries in Cushing's syndrome and those in Addison's disease indicates that in cases of Cushing's syndrome with pituitary adenoma there is probably no overproduction of ACTH by the nonadenomatous part of the gland. In our single case with no pituitary adenoma, there was no histologic evidence of oversecretion of ACTH.

It would appear that both beta and gamma cells are concerned with the production of corticotropin. We regard gamma cells as actively secreting forms with little storage of granules. Beta cells may also be actively secreting, as in beta-cell tumors of Cushing's syndrome, but they store enough granules to permit intense staining. Russell reported that, with the

aldehyde-fuchsin technique, beta and gamma cells show a similar staining reaction, which differs only in intensity (10, 11). There was no evidence in our study linking the delta cells with ACTH secretion. We suggested previously that the delta cell secretes gonadotropin (1). In both Addison's and Cushing's disease, the proportion of delta cells was sometimes greater than expected after a chronic illness. This finding may be related to the variable response of gonadal function to adrenal disorders.

In a previous publication (1) we presented evidence that beta and delta cells were two distinct types of basophils. In the present investigation it has been shown that these cells also respond differently to disturbances in adrenocortical function. Beta cells were the site of Crooke's hyaline change induced by an excess of glucocorticoids, and were consistently reduced in Addison's disease. Delta cells did not show these reactions. Basophil adenomas, with or without Cushing's syndrome, were composed of beta, not delta, cells. Our beta cells seem to correspond to the beta cells of Russell, which showed similar reactions to adrenal disorders (11). These findings are in keeping with the view that the beta cell is distinct from the delta cell.

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DAILY VARIATIONS IN THE THYROIDAL UPTAKE OF I^{131} IN HUMAN SUBJECTS*

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ABSTRACT

Forty-nine euthyroid subjects were studied on two successive days to evaluate the constancy of thyroidal I^{131} uptake. The thyroidal uptake of I^{131} and the urinary excretion of iodide (I^{127}) were measured three and twenty-four hours after giving I^{131} tracers. Six patients had day-to-day changes in 24-hour I^{131} uptake of more than 10 per cent of the administered tracer; 3 had variations of 3-hour I^{131} uptake of more than 6 per cent. No correlation between the variability of the thyroidal uptake of I^{131} and the amount of iodide excreted was found when the daily excretion was less than 500 μg.; when more than this amount was excreted, variability in thyroidal I^{131} uptake from one day to the next was no longer encountered. Thus, when an apparent discrepancy between the clinical picture and the thyroidal uptake of I^{131} is observed, day-to-day variation of this index of thyroid function should be considered among the possible causes of such a finding.

IN THE evaluation of the thyroidal uptake of radioactive iodine (I^{131}) it is generally assumed that if the clinical status of a patient is unchanged this measurement will remain relatively constant. A review of the literature revealed that although unexplained variation of thyroidal I^{131} uptake has been observed (1–5), the frequency, magnitude and clinical importance of this phenomenon has been incompletely studied. A recent investigation (6) concerned with the possible effect of feeding upon 3-hour and 24-hour I^{131} uptakes showed that in 10 per cent of the 40 subjects there were sizable daily variations of uptake, not related to the ingestion of food just prior to the administration of tracer I^{131}. This observation prompted the present study , which was designed to assess the incidence and magnitude of day-to-day variations of thyroidal I^{131} uptake.

CLINICAL MATERIAL AND METHODS

Patients

Forty-nine patients were studied. Twenty-two were chosen from the medical wards of a general hospital and 27 from the medical wards of a Veterans Administration

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Hospital. All patients whose management permitted inclusion in the study were used, except those with known iodine exposure. Three patients who had nontoxic goiters and 1 with a previous history of thyrotoxicosis were included.

Methods

Isotope technics. Oral tracer doses of I^{131}-iodide were given after an overnight fast. At each hospital, its routine technic for I^{131} uptakes on two successive days was followed: The first and second tracer doses of I^{131} were 8 μc. and 24 μc. at one institution and 25 μc. and 50 μc. at the other. A 1/16-inch lead filter was placed over the scintillation crystals to reduce the effect of backscatter from the neck tissue. Appropriate counting of radioactivity was carried out over the neck, thigh, and isotope standards, at three and at twenty-four hours after the ingestion of each tracer dose. Food was withheld each day until after the 3-hour counts were completed. On the second day the residual radioactivity in the thyroid gland from the first tracer dose was deducted from the total neck counts, correcting this residual activity for physical decay only.

Urine collections. The entire 24-hour urine excreted during each test day was collected in iodide-free containers to be analyzed for creatinine and iodide (I^{127}).

Chemical methods. The completeness of each urine collection was validated by determining creatinine excretion by the method of Clark and Thompson (7). Serum protein-bound iodine (PBI) concentration was determined by a modification of the method of Barker *et al.* (8), and urinary iodide by a modification of the perchloric-acid procedure of Zak *et al.* (9). With repeated determinations of iodide excretion, the standard error of the mean was found to be 1.2 μg. for 3-hour specimens and 9.1 μg. for 24-hour specimens

RESULTS

All the patients were clinically euthyroid; this was confirmed by their serum PBI levels, which ranged between 3.5 μg. and 7.4 μg. per 100 ml., corresponding to the usual normal values. PBI concentration was measured on both test days for the first 22 patients. Since no variation greater than 1 μg. per 100 ml. and no statistical difference in the values for the two days was found, only a single PBI determination was performed upon the remainder.

Urine collections were found to be incomplete in 17 patients. For the remaining 32 patients the determination of nonisotopic iodide (I^{127}) was considered a valid measurement.

The data were examined to evaluate the effect of the test procedure on Day 1 upon the results obtained on Day 2. The mean thyroidal I^{131} uptake, its standard deviation, and the variability of I^{131} uptake in the tests of all 49 subjects on Days 1 and 2 did not differ significantly from each other. This seems to exclude the possibility that the results of the second day's test were influenced by the test performed upon the previous day.

Figure 1 relates the levels of 24-hour thyroidal I^{131} uptakes on two successive days. Although the majority showed little variation, among 29 patients whose uptakes were below 30 per cent on both days, only 1 had a day-to-day variation exceeding 7 per cent of the administered tracer dose

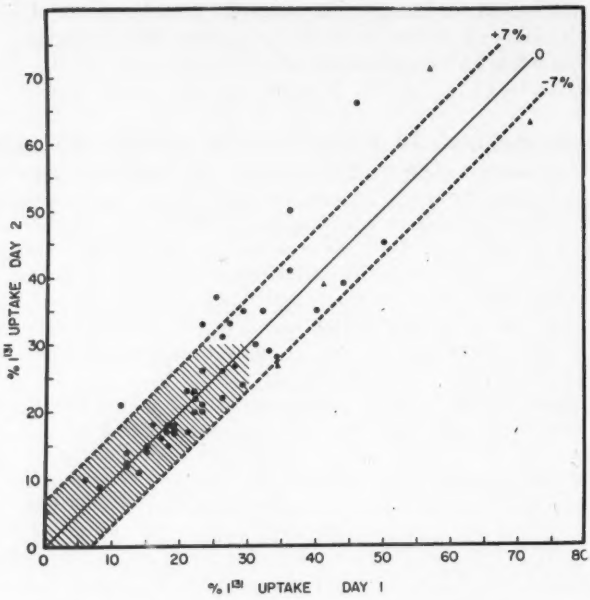

FIG. 1. 24-Hour thyroidal I^{131} uptake on two successive days. The uptakes are plotted as a percentage of the administered tracer dose of I^{131}. The "O" line is drawn to show where the values would fall if the uptakes were the same on both days. The dashed lines delimit day-to-day variation less than 7% of the administered tracer, and the hatched area includes uptakes less than 30% that varied less than 7%. The results in the patients with known thyroid disease are identified with triangles; those in the remaining patients, with circles.

of I^{131}; among the 20 patients with one or both uptakes above 30 per cent, 6 showed variations of 9 per cent or more of the administered tracer dose. Two of the 3 patients with nontoxic goiters and 1 with previous thyrotoxicosis were among the 6 subjects showing a day-to-day variability greater than 10 per cent of the administered dose.

In Figure 2 the mean 24-hour thyroidal I^{131} uptakes and day-to-day variations of I^{131} uptake are compared with the mean daily excretion of iodide. A wide range of daily iodide excretion was found—96 μg. to 1190 μg. per day. When the average daily iodide excretion was less than 500 μg. no consistent relation was apparent, but when the iodide excretion exceeded this amount no patient had a 24-hour thyroidal I^{131} uptake greater than 20 per cent of the administered dose, nor did the uptakes on two successive days vary more than 3 per cent of the tracer dose. This was less than the variation in thyroidal I^{131} uptake observed in the patients whose uptakes were also below 20 per cent, but whose mean daily iodide excretion

was less than 500 μg. No consistent relationship was observed between day-to-day changes in I^{131} uptake and changes in iodide excretion on those days.

The data for the 3-hour thyroidal I^{131} uptakes and urinary iodide excretions revealed trends similar to those for the 24-hour measurements. Although most values varied little from one day to the next, 3 patients showed variations of considerable magnitude, namely, 6, 10 and 13 per cent of the administered I^{131}. The 6 per cent variation occurred in the patient with treated thyrotoxicosis whose 24-hour variation was 7 per cent; in the other 2 patients there was no evidence of thyroid disease.

DISCUSSION

The results of the present study indicate that in approximately 10 per cent of persons there is sufficient variation of the thyroidal uptake of I^{131} from one day to the next to be of potential clinical importance. This is illustrated by 3 of the patients tested, whose thyroidal I^{131} uptakes on one day were in the normal range, but on the other day suggested hypothyroidism in 1 patient and hyperthyroidism in the other 2. The few patients with known thyroid disorders who were tested seemed particularly prone to exhibit variations in I^{131} uptake. Whether or not thyroid dis-

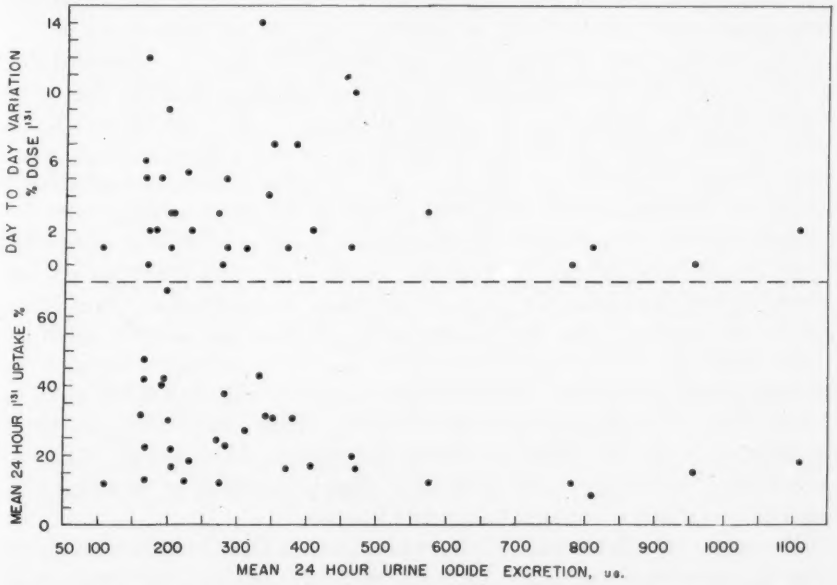

FIG. 2. Day-to-day variation of 24-hour thyroidal I^{131} uptake and mean 24-hour I^{131} uptake compared with mean daily iodide excretion.

orders tend to cause more variability in the results of this test seems worthy of further study.

Estimation of thyroidal reserve by stimulating the thyroid gland with thyrotropin (TSH), as suggested by Jefferies and co-workers (10), compares 3-hour I^{131} uptakes and PBI levels on two successive days, before and after the administration of TSH. In the present study, 3 patients showed a sizable variation between two successive 3-hour uptakes. The variations were of sufficient magnitude to have mimicked the effect of TSH. This finding emphasizes the importance of determining the response of the PBI level as well as the response of the I^{131} uptake to administration of TSH in the assessment of thyroid reserve.

The present study does not identify the cause of the observed differences in thyroidal I^{131} uptakes in the same person on two successive days. An attractive explanation would be that a change in the amount of stable iodine (I^{127}) in the diet would so alter the specific activity of the I^{131} presented to the thyroid gland that the uptake of the isotope might change, although the quantity of iodide trapped would remain the same. The lack of correlation between changes in I^{131} uptake and changes in iodide (I^{127}) excretion on the two successive test days do not support this hypothesis, even though it is well established that over longer periods euthyroid persons exhibit an inverse relationship between iodide intake and thyroidal I^{131} uptake.

Consideration was next given to the possibility that the observed differences might be due to changes in thyroidal trapping of iodide that were sufficient to be reflected in the observed change in 24-hour I^{131} uptake. A change of 10 per cent in the thyroidal clearance of I^{131} from one day to the next (assuming that renal clearance of the isotope remains unchanged) would result in relatively small alterations in the 24-hour thyroidal I^{131} uptake, reaching a potential maximum of 4.5 per cent change if the 24-hour uptake were 60 per cent of the tracer dose. The effect on uptake would be less than this, both above and below this level of uptake. Thus, one would expect large daily fluctuations in the rate of thyroidal I^{131} trapping in some of the subjects tested if this were the explanation of the observed results. After the present study had been concluded, the work of Francois and his colleagues was reported (11). They observed considerable fluctuation in the thyroidal uptake of radioiodine when the same person was tested repeatedly, and concluded that this might be explained by sizable spontaneous changes in thyroid function.

The results of the serum PBI determinations in the 22 patients in whom this measurement was made on both test days support the findings of others (12, 13) that this measure of thyroid function is relatively constant over a short period. The determination of thyroidal uptake of I^{131}, on the

other hand, seems to be more variable, and this should be taken into account in studies involving the comparison of successive measurements of this parameter of thyroid function.

Acknowledgment

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IODINE METABOLISM IN GOITROUS CRETINS*†‡

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ABSTRACT

The iodine metabolism of 6 sporadic goitrous cretins was studied by determinations of stable and radioactive iodine. The 6 patients were placed in 3 groups on the basis of the level and type of metabolic block in the chain of events leading to the elaboration of thyroid hormone. The 3 patients in Group 1 were able to trap iodide without being able to bind it to protein, although in 1 patient minute amounts of monoiodotyrosine were found in the thyroid gland. The 2 patients in Group 2 could trap iodide and totally bind it to protein, but the resulting iodotyrosines were converted to only a small amount of thyroxine. Radioiodinated tyrosines were present in the serum and urine of these 2 patients. The single patient in Group 3 was able to trap iodide but, unlike those in Group 2, could bind only part of it to protein. The resulting thyroidal iodotyrosines were associated with only a small amount of thyroxine in the thyroid gland and with iodotyrosines in the urine.

IT IS generally assumed that a number of thyroidal enzymes are required to withdraw inorganic iodide from the blood and return thyroxine to it. Patients with sporadic goitrous cretinism have been described who seem to lack 3 of these enzymes: *a*) those with a thiouracil type of defect—ability to concentrate iodide in the thyroid gland but inability to combine it with protein (1–5); *b*) those who produce monoiodotyrosyl and diiodotyrosyl but in whom there is an impaired coupling enzyme so that insufficient thyroxine is synthesized (3); and *c*) those in whom all the steps through production of thyroglobulin and its proteasic breakdown are believed to be normal, but in whom the iodotyrosine deiodase ("deshalogenase") is deficient; apparently this is the largest single group. The result is a con-

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tinous loss from the body of monoiodotyrosine and diiodotyrosine (6–15).

Although the number of sporadic goitrous cretins described in the medical literature exceeds 60, only a limited number of cases have been analyzed in detail with radioiodine (1–19). From the latter group have emerged the afore-mentioned 3 types of enzymic disorders, each with its apparently typical radioiodine pattern; many of these cases have been presented in tabular fashion by Lelong and associates (5).

We recently had the opportunity to study 6 institutionalized goitrous cretins. The thyroid gland, serum and urine of these cretins were analyzed in detail for radioiodine. Difficulty was encountered in attempting to place these 6 cretins into a rigid system of classification.

MATERIAL AND METHOD

The 6 goitrous cretins were carefully selected on the basis of their history and clinical appearance. All thyroid therapy was stopped at least two months prior to the beginning of this study. Carrier-free sodium iodide^{131} was given intravenously in doses of 50 to 500 microcuries.[1]

Routine procedures included determination of the basal metabolic rate, assays of stable iodine in the serum, determinations of plasma cholesterol and serum carotene, x-ray examination of the right hand and wrist for bone age, and photographing of the face and thyroid region.

Special procedures included:

1. Measurement of uptake of I^{131} by the thyroid gland according to a previously described method (20).

2. Determination of the thyroidal and renal clearances of plasma I^{131} by a local adaptation (20) of the method of Berson and associates (21).

3. Serum assays of I^{131} (22) to ascertain the levels of total serum I^{131}, protein-bound I^{131} (PBI^{131}), and butanol-insoluble I^{131} (BII^{131}). These assays were interpreted as follows: total serum I^{131} minus PBI^{131} = inorganic iodide^{131}; BII^{131} = thyroglobulin or any other butanol-insoluble radioiodinated compound; PBI^{131} minus BII^{131} = radioiodinated thyronines and tyrosines. Control studies with diiodotyrosine I^{131} and monoiodotyrosine I^{131} added to nonradioactive serum revealed that at least 97 per cent of either was extractable by acid butanol.

4. Delineation of thyroid glands by a scintillation scanning technic (23, 24).

5. Histologic and autoradiographic study of biopsy specimens from the thyroid. In Cases 1, 2, 4 and 5, slices of formalin-fixed tissues were used to make autoradiograms by the contact method (25). In Cases 3 and 6, freeze-dry fixed tissue, prepared by a modification of Stowell's (26) apparatus and technic, was utilized; the autoradiograms were then made before and after floating the freeze-dry tissue in warm water for thirty minutes. This proved to be a relatively simple method of assessing organic binding of iodide^{131}, since inorganic iodide was quickly leached from the floated slices; they could then be compared with the unleached slices.

6. Column chromatographic study of trypsin hydrolysates of saline extracts of radioiodinated thyroid gland obtained at biopsy, as well as butanol extracts of urine and acid-butanol extracts of serum. The method was a continuously recording modifica-

[1] The I^{131} was supplied by the United States Atomic Energy Commission.

tion (22) of the method of Braasch and associates (27). Unlike paper chromatographic technics, quantitation is simple, accurate and reproducible for iodide and all the iodinated amino acids except thyroxine.

7. Ascending paper chromatographic study using the conventional butanol-dioxane-ammonia system (28). This technic was resorted to primarily because of the uncertainty of defining thyroxine by the column chromatographic method. Thyroxine appears at the solvent front of the column along with unidentified substances which are possibly artifactitious. Since the solvent-front unknown of the columns also migrates to the solvent front of the paper strips, it is none of the known iodothyronines, iodotyrosines or iodide, and can thus be clearly distinguished from thyroxine.

8. Intravenous administration of sodium thiocyanate (1 Gm.), to evaluate organic binding of thyroidal iodide^{131} (29). This was accomplished by *in vivo* measurements over the thyroid gland and by assays of serum I^{131} before and after injection of the thiocyanate.

The procedure followed with each case was that of 1) clinical evaluation, 2) obtaining the routine laboratory data and photograph, 3) determining the uptake of I^{131} and the thyroidal and renal clearances, and the assaying of serum I^{131} after a 50-microcurie dose of I^{131}, 4) obtaining a scintillation scan of the thyroid gland, biopsy specimen of the thyroid, autoradiogram, histologic examination, serum and urine analyses after a dose of about 500 microcuries of I^{131}, and 5) repeating the thyroidal and serum measurements of I^{131} after a dose of 50 microcuries of I^{131} followed by administration of sodium thiocyanate.

RESULTS

The results obtained in the 6 cases are tabulated. Table 1 lists the clinical data, Table 2 the prebiopsy I^{131} data, Table 3 the chromatographic results, and Table 4 the effects of sodium thiocyanate.

On the basis of the serum protein-bound iodine (PBI) value alone, it is possible to divide the 6 cases into 2 groups: Group 1, consisting of Cases 1 to 3 in which the values for PBI in the serum were less than 1 microgram (μg.) per 100 ml.; and Group 2, consisting of Cases 4 to 6 in which the values for PBI were low borderline. As will be discussed later, Case 6 seemed different from Cases 4 and 5, and Case 1 different from Cases 2 and 3. On other grounds, Cases 1, 2 and 3 seemed to have one common defect, and Cases 4 and 5 a different one. These cases will therefore be presented in 3 groups: Group 1 (Cases 1, 2 and 3), Group 2 (Cases 4 and 5), and Group 3 (Case 6).

Group 1—Case 1 was that of a young male (Fig. 1a) with an intelligence rating of imbecile, who had experienced some general improvement from thyroid therapy although he was still mentally and physically retarded. He had a palpable nodule in the left lobe of his thyroid.

Cases 2 and 3 were those of a brother and sister (Fig. 1b and c) with intelligence ratings of idiot; they reportedly had another cretinoid sibling. Both patients had had large diffuse goiters but the sister had undergone thyroidectomy two years previously; since only a nodule measuring 2 by 2 cm. remained in her neck, I^{131} studies were less satisfactory than in her brother.

TABLE 1. SUMMARY OF CLINICAL AND LABORATORY FINDINGS IN 6 CASES OF SPORADIC GOITROUS CRETINISM*

Patient			Clinical diagnosis of thyroid status	BMR† (%)	Serum PBI‡	Serum BEI§	Plasma cholesterol‖	Serum carotene¶	Bone age (yrs.)
Case No.	Age & Sex	Height (ins.)							
1	17 M	62	Severely hypothyroid with goiter	−25	0.4	Not det'd	293	285	14
2	31 M	62½	Severely hypothyroid with goiter	−20	0.7	Not det'd	267	543	Adult
3	22 F	48	Severely hypothyroid with small remnant of thyroid in neck	−37	0.8	Not det'd	438	565	<15
4	61 F	48½	Euthyroid with goiter	−17	4.4	3.9	200	160	Adult
5	52 F	52	Euthyroid with goiter	− 5	4.1	3.7	217	142	Adult
6	49 M	55	Mildly hypothyroid with goiter	Not det'd	3.1	2.1	155	63	Adult

* Patients 2 and 3 were siblings, and Patients 4 and 5 were siblings.
† BMR = basal metabolic rate; normal values = −15 to +15 per cent.
‡ PBI = protein-bound iodine; normal values = 3.5 to 7.5 micrograms per 100 ml.
§ BEI = butanol-extractable iodine; normal values = 3.0 to 7.0 micrograms per 100 ml.
‖ Normal values for plasma cholesterol = 150 to 250 mg. per 100 ml.
¶ Normal values for serum carotene = 125 to 150 international units per 100 ml.

TABLE 2. SUMMARY OF PRELIMINARY DETERMINATIONS OF RADIOIODINE*

Case	Peak thyroidal uptake of I^{131}	Apparent thyroidal clearance of I^{131} (ml./min.)	Renal clearance of I^{131} (ml./min.)	Assays of serum I^{131} 24 hrs. after dose (% dose/liter)	
				I^{131}	PBI^{131}
1	57% in 28 min.	231	23	1.28	.09†
2	59% in 15 min.	253	26	.61	.04†
3	14% in 3 min.		16	1.68	.14†
4	19% in 23 hrs.	10	16	.61	.33
5	19% in 24 hrs.	9	43	.74	.53
6	36% in 6 hrs.	100	25	.85	.17

* Normal values (18):
Thyroidal uptake: 12 to 47 per cent, peak at 24 hrs.
Thyroidal clearance: up to 26 ml./min.
Renal clearance (19): 11 to 58 ml./min.
Serum PBI^{131} level at 24 hrs.: 0.014 to 0.167 per cent of dose per liter.

† Values below 7.5 per cent of the iodide concentration are considered to represent merely iodide carried down with the protein precipitate; the apparent PBI^{131} level in Case 3 was just over this arbitrary limit.

TABLE 3. COLUMN CHROMATOGRAPHIC DATA ON EXTRACTS OF THYROID HYDROLYSATES, SERUM AND URINE

Case	Extract	Hours after dose of I^{131}	Per cent of total I^{131} in:				
			Solvent front*		Iodide	DIT	MIT
			Total	T_4			
1	Thyroid adenoma	25	11.7	0	88.3	0	0
	Thyroid parenchyma	25	10.4	0	88.6	0	1.0
	Serum	26	59.1	0	40.8	0	0
	Urine	96	0.8	0	99.2	0	0
2	Thyroid	5	8.5	0	91.5	0	0
	Serum	24	42.6	0	57.4	0	0
	Urine	23	0.8		99.2		
		48	0.7	0	99.3	0	0
3	Thyroid	5	1.4	0	98.6	0	0
	Serum	4½	45.4	0	54.6	0	0
	Urine	25	3.1	0	96.6	0	0.3
4	Thyroid	32	20.0	+†	17.3	39.5	20.7
	Serum	2	69.8	0	30.2	0	0
		7	40.2	?	55.2	2.0	2.6
		12	30.2	?	65.9	1.3	2.6
		24	57.8	trace	34.5	2.5	5.2
	Urine	27	3.2	0	66.7	9.7	20.4
		51	9.2	0	54.4	12.1	24.3
		90	10.2	0	47.0	8.5	34.3
5	Thyroid, left lobe	78	25.9–26.2	—‡	9.2–17.6	22.6–26.5	29.7–42.3
	Thyroid, right lobe	78	9.2–10.7	—	4.8–13.2	20.7–22.6	55.0–63.2
	Serum	11	21.5	—	75.5	0.7	2.3
		23	74.5	—	19.4	2.3	3.8
		47	76.4	—	16.2	1.6	4.5
		71	82.6	—	15.3	0	2.1
	Urine	11	3.7	—	80.6	11.9	3.8
		23	6.6	—	78.0	5.9	9.5
		70	4.6	—	13.3	50.4	31.7
		148	7.1	—	36.5	17.9	38.5
6	Thyroid, left lobe	25	16.8§	+	20.1	15.6	47.5
	Thyroid, right lobe	25	8.2§	+	23.9	18.0	49.9
	Serum	24	46.5	trace	53.5	0	0
		46	39.5	trace	60.5	0	0
	Urine	46	2.8	0	94.7	1.1	1.4

* The solvent front from the column was concentrated and rechromatographed on paper with butanol-dioxane saturated with ammonia. Thyroxine was identified clearly (+), in small amount (trace), or questionably (?).

† Triiodothyronine I^{131} also present; most of the solvent-front I^{131} was neither of the 2 thyronines.

‡ Paper chromatographic determinations were not made in Case 5.

§ Unidentified areas of I^{131} were found between the thyronine area and the solvent front on the paper chromatograms.

TABLE 4. EFFECT OF SODIUM THIOCYANATE ON RADIOACTIVE IODINE IN THE THYROID AND IN SERUM

Case	Effect of sodium thiocyanate on thyroidal I^{131}	Assays of serum I^{131} immediately before NaSCN (% dose/liter)		Minutes after I^{131} that NaSCN was given	Assays of serum I^{131} after NaSCN (% dose/liter)		
		Iodide^{131}	PBI^{131}		Minutes after NaSCN	Iodide^{131}	PBI^{131}
1	Rapid, marked decrease	3.84	.18*	118	17	4.66	.18*
2†	Rapid, marked decrease	2.61	.18*	47	7	5.95	.24*
4	No effect	2.08	.45	371	3	2.09	.44
5	No effect	3.22	.57	400	65	2.92	.47
6‡	Rapid, but moderate decrease	3.62	.22*	36	14	5.62	.25*

* Values below 7.5 per cent of the iodide concentration are considered to be merely iodide carried down with the protein precipitate.

† See Figure 3.

‡ See Figure 9.

These 3 patients followed a common pattern: a very rapid thyroidal uptake of I^{131} for less than half an hour, followed by an exponential disappearance of thyroidal I^{131} which was practically identical in rate with the disappearance of I^{131} from their serum samples (Fig. 2). Probably because of the small size of the gland, only 14 per cent of the dose of I^{131} was identified in the thyroid in Case 3; in the other 2 patients, thyroidal uptake exceeded 50 per cent of the dose, which was reflected by the extremely high thyroidal clearances of plasma I^{131}.

Satisfactory studies of the effect of administration of sodium thiocyanate (1 Gm.) were possible in Cases 1 and 2 only. In both, thyroidal I^{131} decreased precipitously after the injection (Fig. 3) and there was an accompanying rise in the level of serum I^{131}.

The data so far presented do not distinguish Case 1 from Cases 2 and 3. However, there was definite evidence that in Case 1 the patient was able to convert a minute amount of I^{131} to an organic form. Unlike Cases 2 and

a b c

FIG. 1. a. Case 1. b. Case 2. c. Case 3.

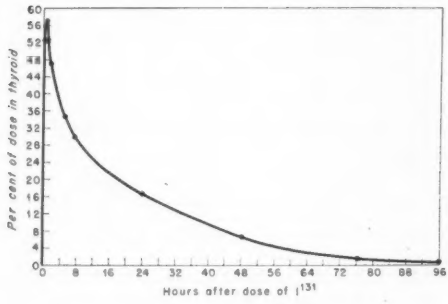

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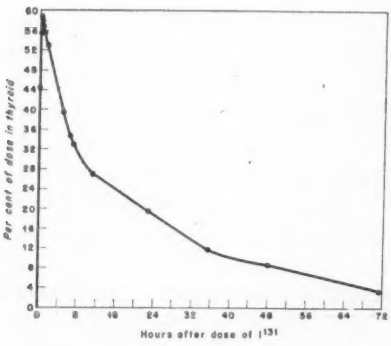
FIG. 2. Thyroidal I^{131} after intravenous dose. a. Case 1. b. Case 2. c. Case 3.



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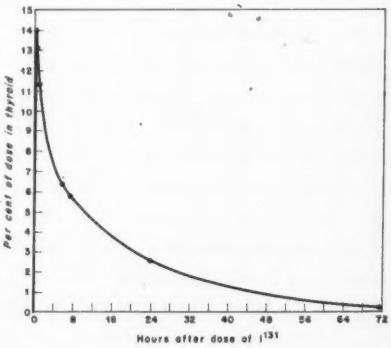

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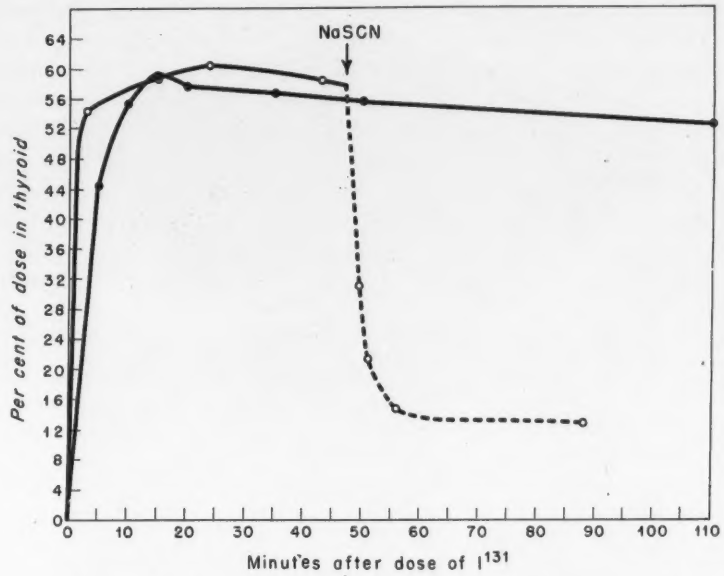

FIG. 3. Case 2. Uptake of radioiodine by the thyroid, showing effect of intravenous administration of sodium thiocyanate. The unlabeled curve is a detail of the first portion of the one shown in Figure 2*b*, repeated here for comparative purposes.

3, in which there was no evidence of any organic radioiodine in any specimen by any technic,[2] in Case 1 there was repeatedly a small amount (about 1 per cent) of noniodide I^{131} in the monoiodotyrosine area of the column chromatographic effluent of the hydrolysates of thyroid tissue. This apparently trivial amount of organic iodine might be ignored, despite its absence in Cases 2 and 3, except that an autoradiogram of formalin-fixed thyroidal tissue also contained I^{131} (Fig. 4). As was clearly demonstrated in Case 2, *no* inorganic iodide^{131} persisted in tissue which had undergone fixation in an aqueous solution of formaldehyde.

One can simply summarize these 3 cases as having a "thiouracil-like" defect. The inability of these patients to produce thyroidal hormone presumably accounted for the enlarged glands and exaggerated uptakes of I^{131}—a reactive thyroid-stimulating-hormone effect.

Group 2—Cases 4 and 5 (Fig. 5) concerned 61- and 54-year-old sisters with intelligence ratings of idiot. Apparently neither had ever received

[2] A minute amount of monoiodotyrosine I^{131} seemed to be present in a single specimen of urine in Case 3 (Table 3).

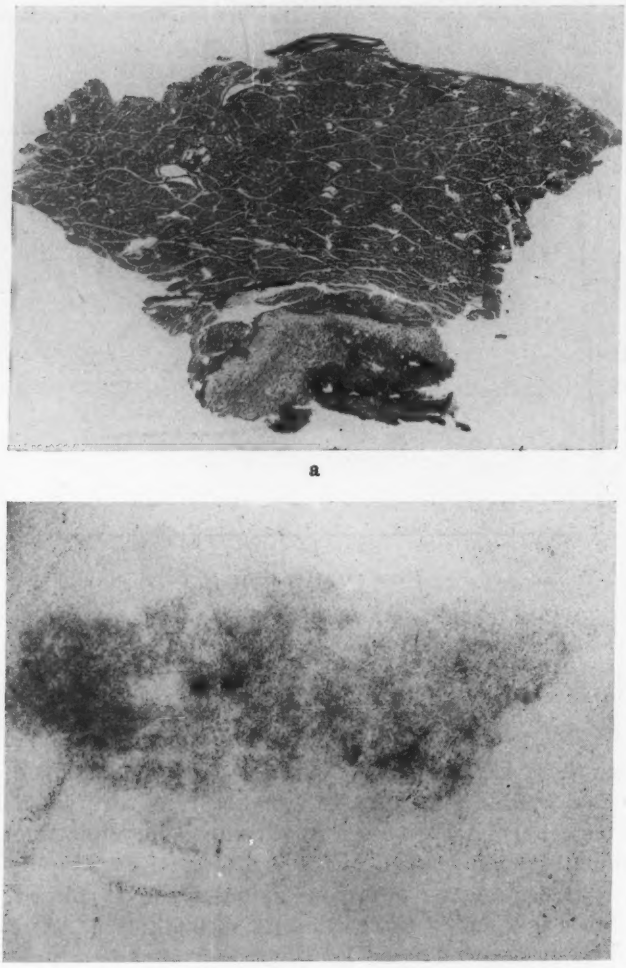

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FIG. 4. Case 1. **a.** Thyroid parenchyma (above) and adenoma (below) (hematoxylin and eosin; ×5). **b.** Autoradiogram of formalin-fixed tissue represented in **a.**

thyroid therapy, probably because neither was frankly myxedematous. Whereas in Case 5 there was a large pendulous adenoma in the right lobe of the thyroid gland, in Case 4 there was only a movable nodule, measuring 3 by 3 cm., in the left lobe of the gland.

The I^{131} data in these 2 cases were practically identical. Both patients took up about one fifth of the dose of I^{131} in their thyroid glands, the peak

being reached in about twenty-four hours, although three-fourths of the uptake was accomplished within the first four hours (Fig. 6). The disappearance of I^{131} from the thyroid gland was accelerated above normal, but not so rapidly as in Cases 1 to 3.

The 24-hour value for serum PBI^{131}, essentially all butanol-extractable, was two to three times greater than the upper limit we have found in normal persons; this resulted in "hyperthyroidal" conversion ratios. The exaggerated values for PBI^{131} were in large part attributable to the presence of radioiodinated monoiodotyrosine (MIT) and diiodotyrosine (DIT) in the serum; neither could be demonstrated in serum two hours after the

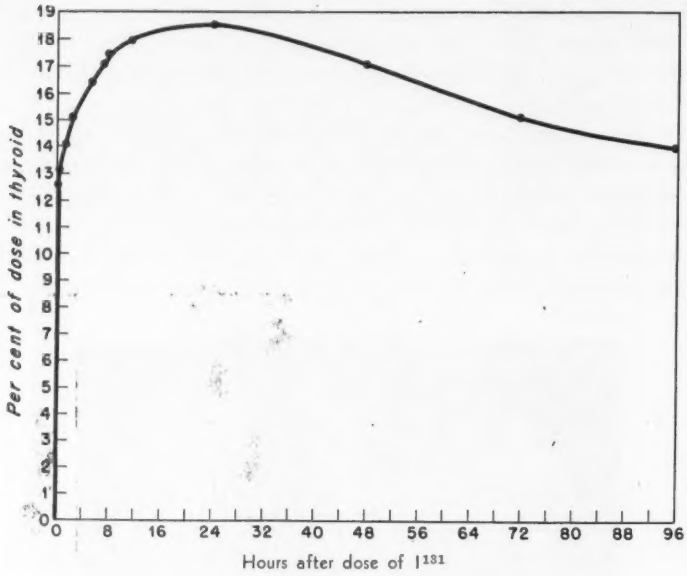
a b

FIG. 5. **a.** Case 4. **b.** Case 5.

dose, but by the seventh to eleventh hours both compounds were present. The highest concentrations found were 5.2 per cent of MIT and 2.5 per cent of DIT at the twenty-fourth hour in Case 4, and 4.5 per cent of MIT and 1.6 per cent of DIT at the forty-seventh hour in Case 5; these percentages represent the fraction of the total serum radioactivity found in each amino acid. Despite the metabolic status of these patients, thyroxine I^{131} could not be demonstrated in the serum in more than "trace" amounts.

As can be seen in Table 3, much of the urinary I^{131} in these 2 patients was radioiodinated tyrosines; in fact after the first twenty-four hours, only half (Case 4) or less (Case 5) was $iodide^{131}$.

The fact that the predominant organic compounds in the thyroid gland were iodinated tyrosines is not in itself abnormal; this also occurs in normal thyroids and in thyroids affected by other disease processes (22, 27). How-



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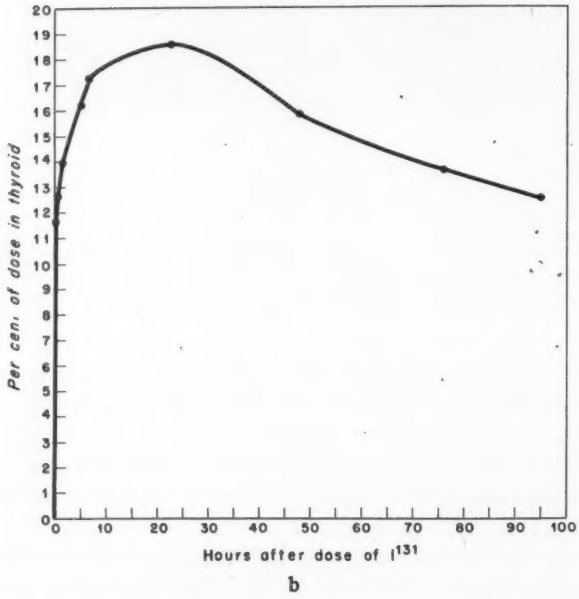

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FIG. 6. Thyroidal I^{131} after intravenous dose. **a.** Case 4. **b.** Case 5.

ever, in Case 4 the amount of identifiable thyroxine was considerably less than normal. Autoradiograms showed evidence of I^{131} after formalin fixation of the tissue, as would be expected from the evidence of organic binding of the I^{131}; that organic conversion was practically complete was ascertained by the lack of any immediate effect of sodium thiocyanate on the concentration of I^{131} in the neck or in the serum.

The 2 patients in Group 2 seem to fall into the class defined by the term "deshalogenase defect," although the relatively modest uptakes of I^{131} by the thyroid glands do not conform to the usually described picture. The 3 patients reported on by Stanbury and his associates (6, 8) had rapid thyroidal uptakes of I^{131}, reaching "hyperthyroidal" peaks within the first

FIG. 7. Case 6.

two or three hours, followed by loss of thyroidal radioiodine three to four times faster than observed in our 2 patients. The values for serum PBI were 0.5 and 2.3 μg. per 100 ml. in 2 of the 3 patients in the series of Stanbury and associates (6, 8) and 4.1 and 4.4 μg. in our 2 patients. The point of real similarity is the finding of iodotyrosines in the serum and urine.

Group 3—The only patient in this group—Case 6 (Fig. 7)—is not placed in either of the other groups because of certain dissimilarities. Somewhat like the patients in Group 1, he had a rapid uptake of I^{131} which reached a peak at about the sixth hour, but the maximal uptake was within the range observed for normal persons (Fig. 8). Like the patients in Group 2, he had values for PBI and BEI that were clearly more than could be achieved by the patients in Group 1. Clinically he was mildly hypothyroid, had an intelligence rating of idiot, and fitted the picture of sporadic goitrous cretinism.

The finding of 1) small amounts of urinary MIT I^{131} and DIT I^{131}

(Table 3), although much less than in the patients of Group 2 and despite the apparent absence of these substances from the serum; 2) the presence of thyroxine I^{131} in the thyroid gland and possibly in the serum; and 3) the modestly accelerated disappearance of I^{131} from the thyroid gland would seem to place this patient in Group 2. From the twenty-fourth hour on, more than half the serum PBI^{131} was not extractable with acidified butanol; this was unique in Case 6 and reminds one of the comparable finding in patients with active Hashimoto's disease (22). The practically instantane-

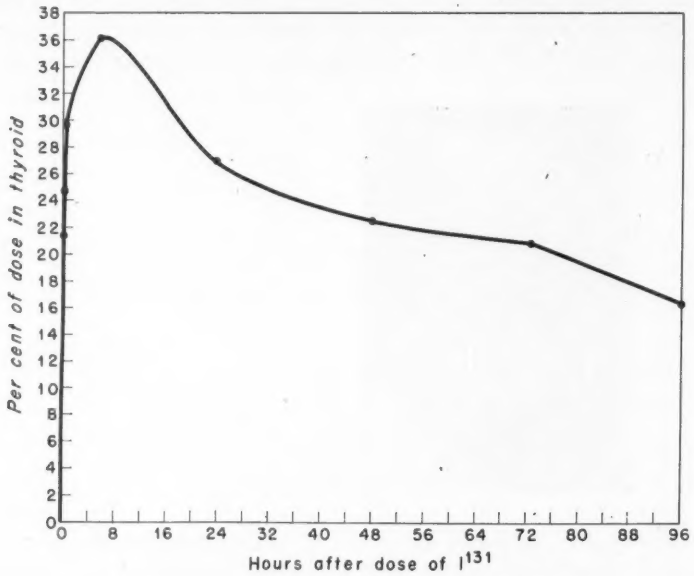


FIG. 8. Case 6. Thyroidal I^{131} after intravenous dose.

ous loss of more than half the thyroidal I^{131} after intravenous administration of thiocyanate (Fig. 9), and the parallel increase in the level of serum $iodide^{131}$, are not in keeping with the "organification" that characterizes the patients of Group 2. If it is thought that the thirty-six minutes allowed was insufficient to permit organic binding of thyroidal I^{131}, it may be pointed out that freeze-dry autoradiograms of the thyroidal biopsy specimen made twenty-five *hours* after a dose of I^{131} also should show a diminution of radioactivity when inorganic $iodide^{131}$ was permitted to leach out of the slices.

The various groups in this study are categorized in Table 5. The $iodide^{131}$ disappeared from the thyroid gland and from the blood at essentially identical rates in each of Cases 1 and 2 and presumably in Case 3. This is

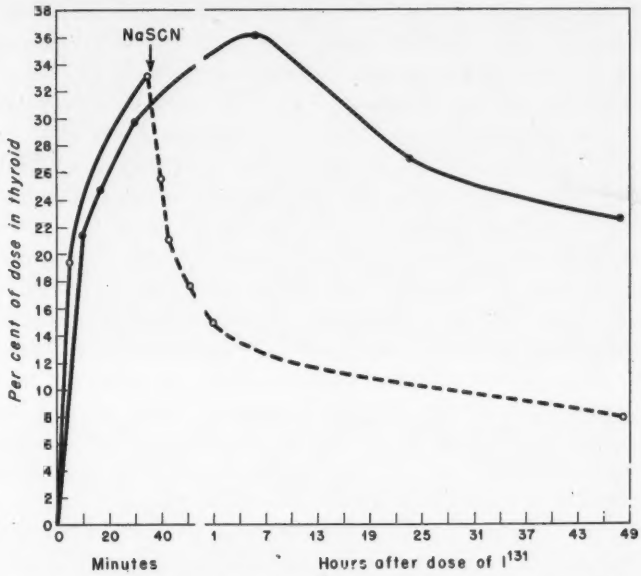


FIG. 9. Case 6. Uptake of radioiodine by the thyroid, showing effect of intravenous administration of sodium thiocyanate. The unlabeled curve is a detail of the first portion of the one shown in Figure 8, repeated here for comparative purposes.

most simply explained by assuming that the thyroidal I^{131} was merely a portion of the iodide I^{131} space of the entire body and was in continuous equilibrium with the blood, which was losing its iodide at a constant rate. In the remaining 3 patients there was a disparity between the disappear-

TABLE 5. RATES OF DISAPPEARANCE OF RADIOIODINE FROM THE THYROID GLAND AND FROM THE SERUM, BASED ON SEMILOGARITHMIC PLOTTING OF THE DATA

Case	I^{131} disappearance rates (%/hr.)	
	From the thyroid	From the blood
1	4.08*	3.96
2	3.85*	4.08
3	4.78*	†
4	0.53	13.9
5	0.38	17.3
6	0.92	3.15

* For the first 4 to 6 hours there was an additional rate of fall of about 70 per cent per hour.

† Insufficient samples for calculation.

ance rates of thyroidal and serum iodide^{131}, reflecting conversion of some thyroidal iodide to noniodide forms. The conversion phenomenon was distinctly less in Case 6 than in Cases 4 and 5.

Pathology—The pathologic findings may be discussed from the standpoint of 1) parenchymal changes, and 2) adenomas or nodules.

In Cases 1 through 4 the thyroid parenchyma was non-nodular, with preservation of lobular architecture. Moderate to marked atrophic changes were observed in all 4 cases, with diminution in size of the lobules and a corresponding increase in connective tissue. These changes were most marked in Case 2 (Fig. 10a), in which small compressed or cordlike lobules with considerable fibrosis were present. The follicles were uniformly small in all 4 cases, were lined by cuboidal epithelium and were virtually empty of colloid. Associated cellular changes included irregularity in size and shape of nuclei with nuclear hyperchromatism. These were present in all 4 cases but especially marked in Case 2 (Fig. 10b). True adenomas were present in all 4 cases. These were predominantly fetal or fetal and solid in architecture, and presented no unusual features (Fig. 10c).

In Case 5 no parenchyma was available for study and the single adenoma removed was of "fetal and colloid" type.

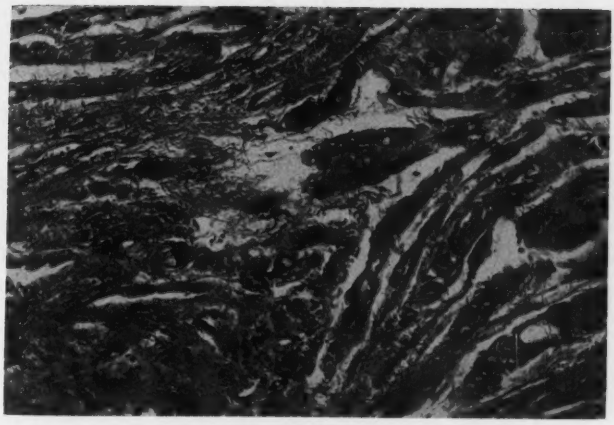
In Case 6 the parenchyma showed considerable nodularity but there were no true adenomas. Numerous macrofollicles were seen, some with focal papillary infoldings and tall epithelium; the remainder of the parenchyma showed a mixture of small empty or normal-sized follicles.

COMMENT

Two of the 6 goitrous cretins presented in this study fall into a previously defined group. Patients 2 and 3, siblings, were able to concentrate I^{131} in their thyroid glands with avidity; however, careful study of serum, urine and thyroidal biopsy specimens, with one possible exception, consistently failed to reveal I^{131} in any form but inorganic iodide^{131}. A congenital, familial defect in the thyroidal conversion of iodide to iodinated amino acids was obviously present. Whether this was the lack of an enzymic catalyst or the presence of some inhibitor was not ascertained.

Patients 4 and 5, also siblings, superficially resembled those described as having a "deshalogenase defect." However, Werner and associates (15) have reported on 1 patient with iodotyrosinuria who did not lack the deiodase of iodotyrosines. Since there was no direct testing of iodotyrosine deiodination, that is, the ability of injected radioiodinated tyrosines to be freed of I^{131}, these patients could be so classed only inferentially.

Despite the impressive array of evidence accumulated by Stanbury and his associates that a "deshalogenase defect" alone is a cause of goitrous cretinism, careful reassessment of the evidence may be worth while. The



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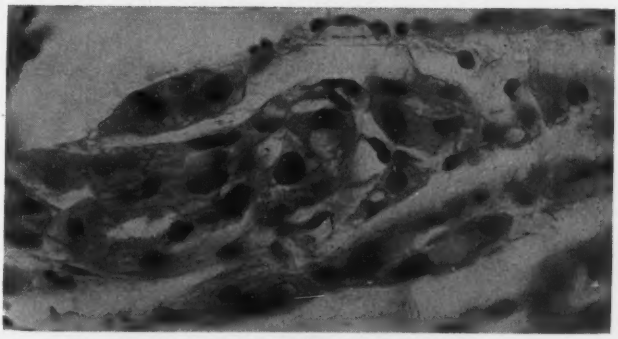

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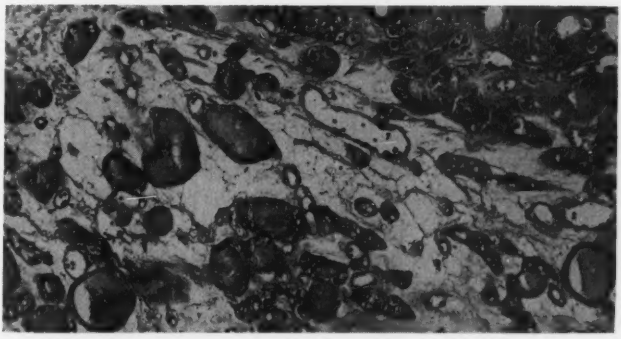

c

FIG. 10. **a.** Case 2. Thyroid parenchyma, showing atrophic lobules composed of cords and strands of follicles and increased fibrosis (hematoxylin and eosin; ×130). **b.** Case 2. High-power view to demonstrate nuclear irregularity and hyperchromatism (hematoxylin and eosin; ×480). **c.** Case 1. Fetal and colloid adenoma (hematoxylin and eosin; ×130).

loss of iodine, as iodotyrosines, from the body cannot of itself account for goitrous cretinism unless insufficient thyroxine is being synthesized and made available to the body tissues. Insufficient thyroxine could result from (a) too small a fraction of the thyroidal iodine being converted into thyroxine even with a compensatory enlargement of the thyroid gland; this has not been claimed by Stanbury and his associates; or from (b) an iodine deficiency as the result of continued loss of iodotyrosine iodine exceeding the intake of iodine; this also had not been claimed by Stanbury's group, although it has by others (13).

If the cretinism represents loss of sufficient iodotyrosines to render the patient iodine-deficient, then the laboratory findings should include those of iodine deficiency, namely, exaggerated uptake of I^{131} by the thyroid and subnormal concentration of iodide in the serum and urine. The former has been reported; in our patients neither was true. The uptake of I^{131} in Cases 4 and 5 was relatively modest; clearances of plasma iodide^{131} were 10 ml. and 9 ml. per minute respectively. In Case 5 the concentration of serum iodide was measured by the indirect method of Stanley (30) based on the relationship,

$$\frac{\text{serum } I^{127}}{\text{serum } I^{131}} = \frac{\text{urinary } I^{127}}{\text{urinary } I^{131}}$$

once equilibrium between the I^{131} and I^{127} is established. In the fasting state the concentration of serum iodide^{127} was found to be 0.59 μg. per 100 ml. of serum, along with a urinary excretion of 124 μg. of iodine in a 24-hour period. In our experience, normal serum values range from 0.2 to 0.5 μg. per 100 ml. of serum by this method. It is therefore difficult to ascribe the metabolic problem in Case 5 to iodine deficiency.

The matter of iodine deficiency could theoretically be settled in a simple manner by administering sufficient iodine to patients lacking the iodotyrosine deiodase to compensate for the urinary loss of iodotyrosine iodine. If Stanbury's concept is correct that the thyroid glands of these patients synthesize thyroxine normally, the serum levels of thyroxine should promptly revert to normal. We believe that the explanation is an oversimplification, and that at least in our Group 2 cases some intrathyroidal defect in synthesis or release of thyroxine is probably associated with lack of iodotyrosine deiodase. Thus, like Werner and colleagues (15) in their case, we were able to find only a small amount of thyroxine I^{131} in the thyroid gland in our Case 4. Similarly in 1 patient reported by Stanbury and colleagues (6), only 3.4 per cent of the thyroidal iodine was in the form of thyroxine.

The remaining 2 cases (Nos. 1 and 6) introduce the possibility that

attempts at categorization may extend beyond biologic reality. The thyroid gland in each of these patients took up I^{131} at hypernormal rates: 231 ml. of plasma cleared of iodide^{131} per minute in Case 1, and 100 ml. per minute in Case 6. In both, more than half the accumulated thyroidal I^{131} was lost when thiocyanate was given intravenously. Yet in both instances there was evidence of the ability to convert some of the iodide to organic forms. In Case 1 the conversion was quantitatively trivial—only a trace of MIT I^{131} could be recovered from the gland and only a small amount of residual radioactivity was demonstrable in slices of thyroidal tissue after fixation in an aqueous medium. Had thyroidal assays not been made, Case 1 could not have been differentiated from Cases 2 and 3.

Case 6 was quantitatively different in that a significant fraction of the accumulated I^{131} was converted into iodotyrosines, a small amount of which was found in the urine, and into thyroxine.

It thus seems that certain patients have a complete inability to process usefully the iodide that has accumulated in the thyroid gland (Cases 2 and 3), that certain patients have a partial ability to synthesize organic compounds (Cases 1 and 6), and that others can convert virtually all their iodide to iodotyrosines but perhaps inefficiently into thyroxine (Cases 4 and 5). The addition of a continuous loss of iodotyrosines from the body (Cases 4, 5 and 6) may be an aggravating factor, particularly during periods of dietary insufficiency of iodine.

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A CONSIDERATION OF THE HYPERCALCIURIA IN SARCOIDOSIS, IDIOPATHIC HYPERCALCIURIA, AND THAT PRODUCED BY VITAMIN D. A NEW SUGGESTION REGARDING CALCIUM METABOLISM*

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ABSTRACT

Some abnormalities of calcium metabolism are considered, with particular reference to the hypercalciuria of sarcoidosis. The results of several metabolic balance studies are discussed. It appears that the 3 disturbances of calcium metabolism in sarcoid—hypercalcemia, hypercalciuria, and increased intestinal absorption—are to some extent independent of each other. In 2 cases of idiopathic hypercalciuria the high urinary calcium excretion was also associated with high intestinal absorption—a state of affairs very similar to that in sarcoidosis when hypercalcemia is not present. In both conditions a negative calcium balance was produced by using a low-calcium diet. The effects of vitamin D on calcium metabolism appear to be mainly the same 3 observed in sarcoidosis. As in sarcoid, evidence is adduced that these 3 effects may be largely independent of each other. It is suggested, however, that in these and in some other conditions (*e.g.*, hyperparathyroidism, acromegaly), the hypercalciuria comes first and is in some way connected with a stimulus to the intestine which leads to an increase in its absorption of calcium. In fact it appears that the intestinal absorption of calcium may be continually governed by the level of urinary calcium in normal people throughout life. This may represent a new principle of body metabolism, with reference to calcium. The disturbances of calcium metabolism in sarcoidosis can logically be explained as the effect of sensitivity to vitamin D, but one difficulty is that not all patients appear to be sensitive. Fortunately, all cases of this condition, whether sensitive to vitamin D or not, appear to respond to cortisone or its analogues.

IT IS well known that sarcoidosis can produce hypercalcemia and hypercalciuria in a certain proportion of cases (estimated at 20–30 per cent in the United States (1), about 10 per cent in London (2), and perhaps 40 per cent in Cape Town). This is important because it may lead

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† Research Bursar, Council for Scientific and Industrial Research.

to renal calculus formation, nephrocalcinosis and renal failure, and to deposition of calcium in the eye (particularly the cornea) and other soft tissues. The cause of this disturbance of calcium metabolism is apparently not secondary hyperparathyroidism, nor generalized destruction of bone, nor sarcoid of the kidneys, nor an excessive binding of calcium to protein in association with hyperglobulinemia (which would not produce hypercalciuria). Albright and his group (3, 4) and Dent and colleagues (5) independently showed that fecal calcium was so low in their patients with sarcoidosis and hypercalcemia that, despite hypercalciuria, calcium balance did not become negative. Both these groups commented on the resemblance of this condition to hypervitaminosis D, and postulated either an overproduction of a calciferol-like substance or an unusual sensitivity to it. Cortisone or its analogues were shown to reduce both the hypercalcemia and the hypercalciuria, and this led Dent to conclude that cortisone might be an antagonist of vitamin D. The idea of excessive sensitivity to vitamin D in some patients with sarcoid is not new, and the production of hypercalcemia with small doses (*e.g.*, 10,000 units a day), or with ultraviolet light, has been described by Scadding (6), Larsson *et al.*(7), Anderson *et al.* (5), Cantwell (8) and Mather (2). An over-absorption of calcium from the gut cannot be the whole story, since both Albright's and Dent's groups realized that the urinary excretion of calcium may often be considerably greater than the total amount of ingested calcium.

In idiopathic hypercalciuria (9) renal stones are formed without any rise in the level of serum calcium. Presumably there must be people with hypercalciuria but without renal stones (*e.g.*, in the early stages of the disorder). However, the normal urinary calcium output is so variable that such a state would be difficult to define. We have seen 3 apparently healthy men whose urinary excretion of calcium was consistently above 400 mg. per day while receiving a normal diet containing roughly 800 mg. of calcium; one of them during four years without any change in habits has remained perfectly well. Such cases could legitimately be termed "idiopathic hypercalciuria without stones." In the developmental stage of osteoporosis there may also be a period of high urinary calcium output without symptomatic or radiologic evidence of bone disease. If discovered in this stage, such cases could be called "idiopathic hypercalciuria without stones but with negative calcium balance." Any patients who are not in a state of negative balance may, like the sarcoid patients, be over-absorbing from the gut by comparison with the normal person.

Other states of hypercalciuria to be mentioned briefly in the light of recent thought are those of acromegaly, hyperparathyroidism, and the effect of vitamin D itself.

METHODS

Five-day metabolic balance studies were performed on 4 patients with sarcoidosis, 1 with idiopathic hypercalciuria and renal stones, 1 with idiopathic hypercalciuria but no renal stones, and 1 with acromegaly. All patients showed hypercalciuria (over 400 mg. per day with a normal intake). Studies on a patient with resistant rickets, and on a normal control Bantu subject are also mentioned. In general technique and charting of results the methods of Reifenstein, Albright and Wells (10) were followed, except that stool markers were not used. Fecal collection periods ran from mid-day to mid-day, and food and urine periods ran from 7 a.m. to 7 a.m. Bowels were kept regular by bland, "non-metabolic" laxatives when necessary, and balance studies were not performed during periods of constipation. Whenever possible, a ten-day period was allowed for equilibration at the beginning of any new regimen before balance studies were performed. This is indicated on the figures by a gap between periods; this gap represents ten days unless otherwise stated. Urinary calcium was estimated by the method of Jackson and Irwin (11); otherwise the chemical methods were those used previously (12).

SARCOIDOSIS

Case 1

A.S. (55/26223), a colored male aged 21 years, complained of three months' vomiting, weakness, loss of weight and polyuria. Examination revealed generalized adenopathy with splenomegaly and episcleral plaques on both conjunctivae. He was afebrile, the erythrocyte sedimentation rate (Westergren) was 25 mm. in one hour and roentgenograms of the chest showed hilar adenopathy. A lymph gland biopsy showed typical sarcoid tubercles.

The concentration of serum calcium ranged from 14 to 16 mg. per 100 ml., urea 75 mg., inorganic phosphorus 4.8 mg., albumin 4.4 Gm., and globulin 4.5 Gm. per 100 ml. Urinary calcium excretion was around 1000 mg. per day.

Unfortunately, after 4 balance periods he discharged himself from hospital.

Balance study (Fig. 1). Four consecutive studies were made—Periods 1 and 4 during intake of a normal diet with about 1 Gm. of calcium in the food, and Periods 2 and 3 during a low-calcium intake of only 120 mg. With the normal diet there was no net loss of calcium from the body despite the enormous urinary output. Fecal calcium was remarkably low, as was fecal phosphorus. Since the normal urine/stool ratio for phosphorus is 2 or 3 to 1, a reduction in fecal phosphorus is not so readily apparent. In this patient, however, the urine/stool ratio was over 10:1, suggesting an over-absorption of phosphorus as well as of calcium. The balances for phosphorus, nitrogen and potassium were all negative.

With the low-calcium intake the urinary and fecal calcium values remained virtually unchanged, so that the balance became grossly negative (some 800 mg. daily). The unavoidable concomitant reduction in phosphorus intake led also to a gross loss of phosphorus; there was no tendency toward compensatory reduction in urinary phosphorus. There was no significant change in serum calcium concentration during the balance periods.

Comment. This study shows clearly the over-absorption of calcium from the bowel, with an exceedingly high urinary excretion, but without any net loss from the body. The calcium output in the urine continued to be extremely high even when the intake was greatly reduced. This would seem to indicate that intestinal over-absorption cannot be the cause of the

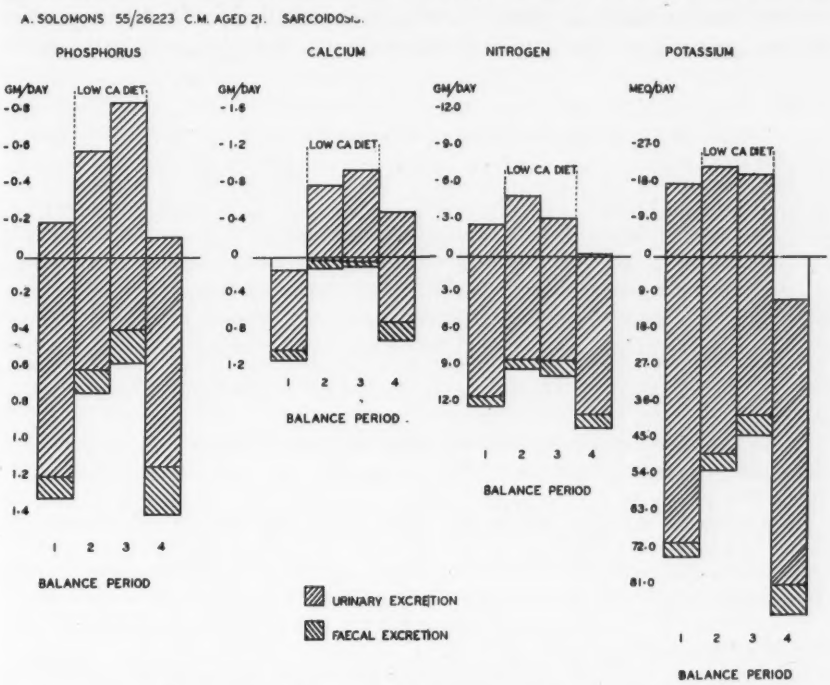


FIG. 1. *Case 1.* Note: 1) the enormous urinary calcium excretion, which did not change with a low-calcium intake, and 2) the extremely low fecal excretion of calcium. There was a similar trend in phosphorus excretion (see text).

As in all figures, the intake is plotted downwards from the baseline, so that any shaded area above the baseline indicates so much negative balance.

hypercalcemia at this stage. In fact the complete lack of any fall in urinary calcium suggests that over-absorption may play no part at all in the production of hypercalciuria, in which case excessive absorption must be a secondary effect—secondary to the hypercalciuria and dependent upon a high calcium intake. However, it may be that a more prolonged study during a period of low-calcium intake would show gradual reduction of calcium in both urine and serum. (Anderson *et al.* (5) found that twenty to forty days of low intake might be required to cause a reduction in the level of serum calcium.) This does not alter the argument, since if over-

absorption were the prime causative factor, a reduction in absorption should produce a quick response in serum and urinary calcium.

The data suggest a concomitant over-absorption of phosphorus, and the lack of depression of urinary phosphorus during periods of low intake further indicates an alteration in phosphorus metabolism. The balance data of Henneman *et al.* (4) also reveal an unusually low fecal phosphorus excretion in their 3 cases, though this fact was not discussed by them.

Case 2

A.C. (56/00577), a plump Bantu girl of 26, complained of cough with white sputum and throbbing in the left eye, which had been present for about a year. She had spent six months in a tuberculosis sanatorium, where no tubercle bacilli were ever found in the sputum and results of the tuberculin test were positive for second strength P.P.D. On admission to the hospital the chest roentgenogram disclosed a generalized reticulated appearance with miliary mottling. Band keratopathy, posterior synechia, nodular iritis and early cataract were evident in the left eye. The urine contained protein and some red blood cells. The erythrocyte sedimentation rate was 120 mm. per hour. The level of hemoglobin was 11 Gm., serum albumin 3.2 Gm., globulin 6.4 Gm., and blood urea 80 mg. per 100 ml., with a urea clearance of 30 per cent. The serum calcium level was 13.0 mg., and inorganic phosphorus 5.2 mg. per 100 ml. Urinary calcium excretion was 550 mg. per day.

Renal biopsy revealed focal round-cell infiltration and tubular atrophy. There were many foci of metastatic calcification in the interstitial tissue, but no sarcoid tubercles were seen.

Prednisone was given for four months, starting with 60 mg. daily and reducing the dosage to a maintenance level of 10–20 mg. daily. There was immediate subjective improvement, rapid reduction of serum and urinary calcium, and complete clearing of the band keratopathy within three months. Unfortunately the iritis became worse, and iris bombé developed with total loss of sight in the left eye. The right eye remained clear. The chest symptoms disappeared.

One year later, no treatment having been given for nine months, the patient felt well but had lost some weight. Sarcoid nodules were seen on the left iris. The chest roentgenogram was now quite clear. The blood urea level was 54 mg. per 100 ml., with a urea clearance of 42 per cent (maximum). Proteinuria was still present but the concentrations of plasma proteins were normal. The level of serum calcium was 9.9 mg. and that of phosphorus, 3.1 mg. per 100 ml. The urinary excretion of calcium was only 56 mg. per twenty-four hours.

Another renal biopsy showed an increase in chronic inflammatory exudate, but less calcification. Occasional sarcoid follicles were seen.

The prednisone therapy appeared to cause a resolution of the pulmonary condition and the disordered calcium metabolism, but normal function could not be restored to the severely damaged kidneys and left eye. It is instructive that sarcoid nodules were found in the kidney at a time when serum and urinary calcium levels were normal, although no nodules had been seen during the hypercalciuric phase. The improvement in renal function appeared to be correlated with the diminution in urinary calcium and

renal calcification, despite the appearance of sarcoid infiltration. This plainly supports the belief that it is the calcium abnormality rather than specific infiltration, which damages renal function (13). However, there has been 1 case report (14) of renal failure with sarcoid infiltration but without disturbance in calcium metabolism.

Balance studies (Fig. 2). Eleven 5-day balance studies were made. During the first 5 periods the effects of different levels of dietary calcium were observed. Serum calcium concentration varied between 11.3 and 13.8 mg. per 100 ml. Both high and low values were recorded during the period in which calcium intake was over 2 Gm. a day. The period of low-calcium intake (104 mg. daily) was continued for fifteen days. At the end of this time the serum calcium level had dropped to a normal value of 10.5 mg. per 100 ml. The serum inorganic phosphorus level ranged between 4 and 5 mg. per 100 ml., but showed no particular trend.

With a normal calcium intake the urinary calcium was about 400 mg. per day, with the high intake it averaged over 500 mg., and with the very low intake it fell to 260 mg. A distinctly negative balance was found only during the latter regimen, when even after fifteen days the urinary calcium greatly exceeded the total intake. Phosphorus balance was slightly positive, except during the low-intake periods, but it was unremarkable.

Vitamin D (calciferol) in a dosage of 100,000 units (2½ mg.) per day, was given for a total of eleven days, during the last five of which a balance study was performed. This drug did not upset the patient and produced no increase in urinary calcium. The last serum calcium level during vitamin D therapy was 11.7 mg. per 100 ml. Fecal calcium and phosphorus were reduced considerably below control levels.

Sodium phytate (supplied by E. R. Squibb & Sons, New Brunswick, N. J.) in the form of a 15 per cent aqueous solution, was given in a dosage of 9 Gm. per day (divided into 6 doses), for two 5-day periods. This substance has the property of binding calcium in the gut (15). A further reduction of urinary calcium was produced, strangely without any rise in fecal excretion, so that the balance became strongly positive. The phosphorus balance became markedly positive, with a low fecal level, indicating complete absorption of the ingested phosphorus of the phytate. There was no subjective improvement. The phytate was not well tolerated; it caused nausea and looseness of the bowels.

Prednisone was then given. The effect was a further gradual drop in urinary calcium, and rapid normalization of the serum levels, which have never since exceeded 11.2 mg. per 100 ml. (this level was found only once, all other recordings being below 10.8 mg.). Fecal calcium rose immediately. After a month's treatment it was higher than the intake, while the urinary calcium was around 100 mg. per day. Fecal phosphorus also rose during

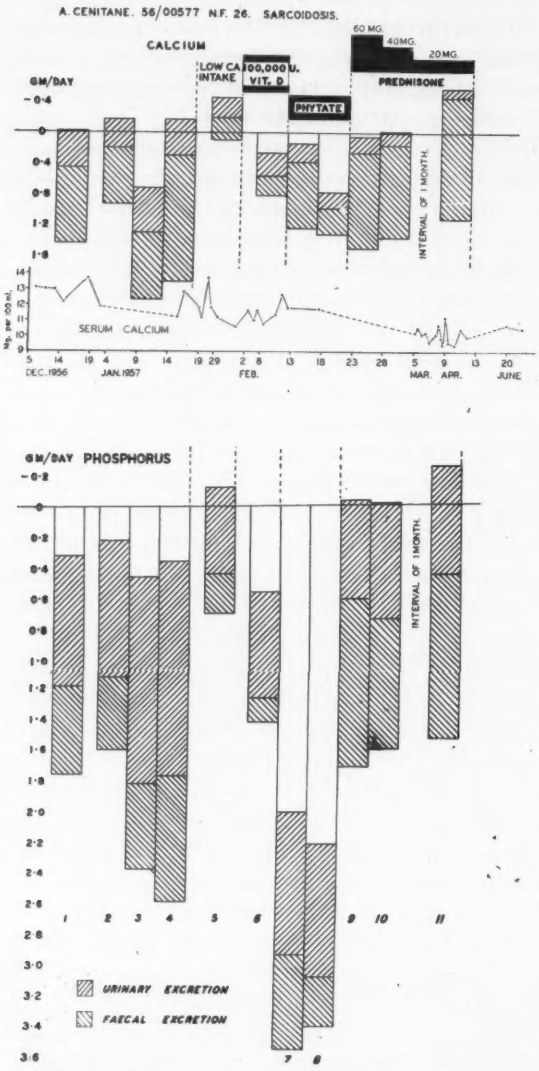


FIG. 2. *Case 2.* Note high urinary calcium, but negative balance only during low intake; lack of effect of vitamin D; only partial benefit from phytate; and rapid improvement in urine and serum calcium levels during prednisone therapy (see text). (Spaces between adjacent balance periods in all figures indicate 10-day intervals during which equilibration under new regimen was taking place, unless otherwise noted.)

prednisone therapy, but there was little change in urinary values. The phosphorus balance became negative.

Comment. The general pattern of balance results was similar to that in Case 1, but the hypercalciuria was less gross. Again, a net loss of body calcium was seen only during the period of low intake. Thus a low-calcium intake did not commend itself as good definitive treatment; moreover it was difficult to maintain and resulted in only a moderate reduction of urinary calcium. Again, the high level of urinary calcium excretion during periods of low intake indicates a loss which could not be occasioned by an intestinal factor.

The lack of excessive sensitivity to large doses of calciferol was noteworthy. A reduction of fecal calcium and phosphorus appeared to be the only effect (with a doubtfully significant slight rise in the level of serum calcium).

Phytate appeared to be helpful in reducing urinary calcium, but it would have been difficult to continue this drug for long. The considerable net retention of calcium and phosphorus during this period was unexpected; the high absorption of calcium and phosphorus may well have been related to a continued effect of the preceding calciferol.

Prednisone was rapidly effective in restoring a more normal calcium status, with an immediate rise in fecal calcium and phosphorus. One drawback to its continued use was the negative balance, which was present after a month's treatment. Any possible ill effects of this condition could presumably be mitigated by using intermittent courses when necessary.

Case 3

N.N. (55/01487) was a 33-year-old Bantu man who worked in a military camp, out in the sun. He first presented with a nodular rash on his face and trunk. He was found to have an old bilateral uveitis which was compatible with a sarcoid etiology. He was otherwise well; his chest was clear. Biopsy of a skin lesion showed typical sarcoid tissue, and the rash rapidly cleared during treatment with oral hydrocortisone. Two months later it reappeared, in association with some loss of weight and polyuria. The erythrocyte sedimentation rate was 8 mm. per hour. The level of serum albumin was 3.6 Gm., and globulin 3.9 Gm. per 100 ml. Urinary calcium averaged 600 mg. per day for three days, yet the serum calcium level was only 10.4 mg. per 100 ml.

During the whole period of observation, 26 determinations of serum calcium were made and all values were well within our normal range. Serum phosphorus and urea levels were also normal.

Renal biopsy revealed several foci of typical sarcoid tissue and the tubules throughout the microscopic section showed pronounced hyaline droplet formation. No calcification was seen.

The cutaneous lesions again rapidly cleared during treatment with hydrocortisone. Urinary calcium excretion became normal, and treatment was discontinued. A year later bilateral parotid enlargement developed, without other evidence of deterioration in the patient's condition, but subsided spontaneously.

Balance studies (Fig. 3). The study was divided into 13 periods. In the control periods the patient chose a diet containing 500 mg. of calcium a day; this resulted in a slightly negative balance. Urinary calcium was in excess of fecal calcium except when the intake was increased to 1.2 Gm. per day. Vitamin D (50,000 to 200,000 units daily) was given for fifteen days, in Periods 8, 9, and 10, and the calcium intake remained constant. There was no effect on calcium, phosphorus, nitrogen or potassium

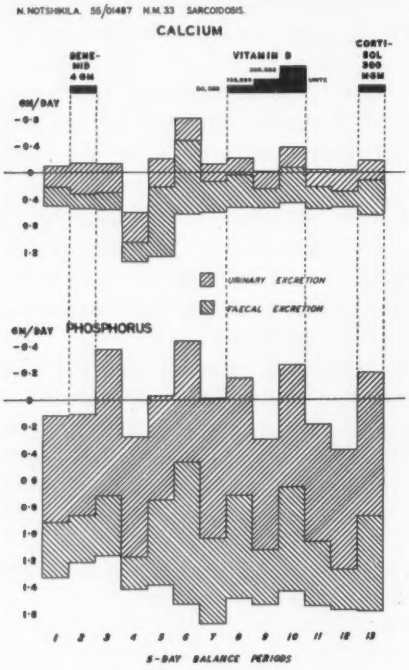


FIG. 3. *Case 3.* Note continued hypercalciuria (despite normocalcemia), slight negative calcium balance but low fecal calcium. Administration of Benemid (part of another experiment) may have increased urinary calcium and phosphorus excretion. Vitamin D had no effect. Hydrocortisone appears to have increased fecal calcium and urinary phosphorus; it also increased urinary nitrogen and potassium (not shown here) (see text).

balance. Hydrocortisone, 300 mg. a day, was given in Period 13. Unfortunately balance studies could not be continued beyond that point. There was an immediate increase in fecal calcium (though no greater than in the last calciferol period (No. 11)) and in urinary phosphorus, potassium and nitrogen. The urinary calcium did not fall immediately, but within another two weeks it had reached a normal level.

Comment. Of major interest in this case was the presence of hypercalciuria without hypercalcemia, yet with the usual over-absorption from the gut as indicated by the low fecal calcium. In this case, at least, hypercalciuria could not have been caused by hypercalcemia, and therefore not by the increased intestinal absorption. Hence these 2 phenomena of over-absorption and over-excretion appear to be unrelated, if it be assumed

that it is the former which can cause the latter (discussed later). Hypercalciuria without hypercalcemia can occur in idiopathic hypercalciuria, and is not at all rare in sarcoid. Thus 3 of Scholtz and Keating's cases (16) with renal complications had normal concentrations of serum calcium, but all had renal calculi.

Case 4

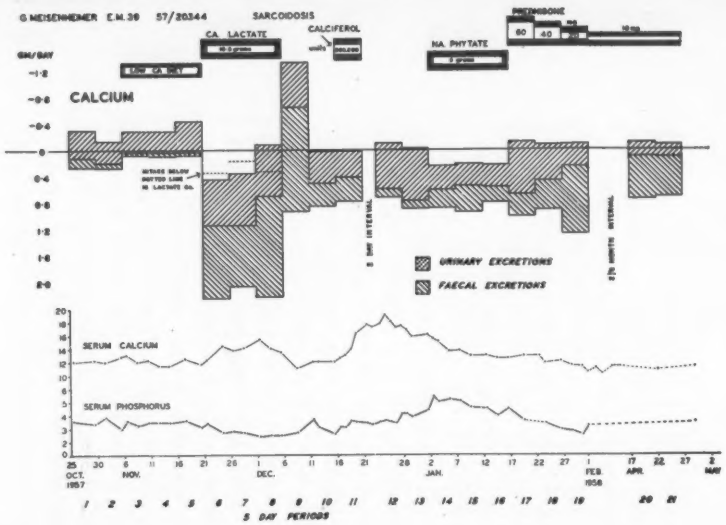
G.M. (57/20344) was a 39-year-old male colored school teacher who complained of tiredness, dry cough and dyspnea of nine months' duration, and more recent weight loss.

He was a lean, healthy-looking man, with generalized crepitations in the lungs and hepatosplenomegaly. Hemoglobin concentration was 13.5 Gm. per 100 ml. The erythrocyte sedimentation rate was 33 mm. per hour (Westergren). X-ray examination of the chest showed fine mottling throughout the lung fields. The level of blood urea was 26 mg., serum albumin 3.9 Gm., globulin 2.9 Gm., calcium 12.5 mg., and inorganic phosphorus 3.6 mg. per 100 ml. The 24-hour urinary excretion of calcium was 880 mg. The diagnosis of sarcoidosis was confirmed by characteristic liver biopsy findings. Renal biopsy failed.

While in the hospital his symptoms improved. The improvement was particularly rapid within a few days after the start of prednisone therapy. The chest roentgenogram showed completely clear lungs within ten days, and the serum calcium level fell to normal within fifteen days.

Balance studies (Fig. 4). Twenty-one balance periods were completed, the last 5 being satisfactorily undertaken while the patient was back at home. The ordered milk supplement was accidentally omitted during the first 2 periods, so that the diet was fairly low in calcium. During these and the real "low-calcium" periods, the calcium balance was negative, and urinary calcium excretion was about 500 mg. a day. Thus hypercalciuria was maintained for twenty-five days during a period of low intake without any decrease, nor did the serum calcium level change. With a high-calcium intake (15.3 Gm. of calcium lactate supplement daily) a positive balance was obtained (though offset by high fecal loss in the period which immediately followed). The urinary calcium increased to 700 mg., being little greater than in control periods Nos. 9, 10 and 13. During the periods of high-calcium intake (Nos. 6, 7 and 8) the serum calcium level rose to 15 mg. per 100 ml., but fell in the following control periods (Nos. 9 and 10).

Because of the lack of response to vitamin D shown by the previous 2 patients, we studied its effect in this patient also. Within one day of administration the serum calcium level had started to rise, and to our consternation it continued to rise (to 18 mg. per 100 ml.) after withdrawal of calciferol. The patient was anorexic and nauseated at this time, but he recovered completely during the next few days without specific therapy. The urinary excretion of calcium did not rise, nor was there any further reduction in fecal calcium at this time, although fecal calcium became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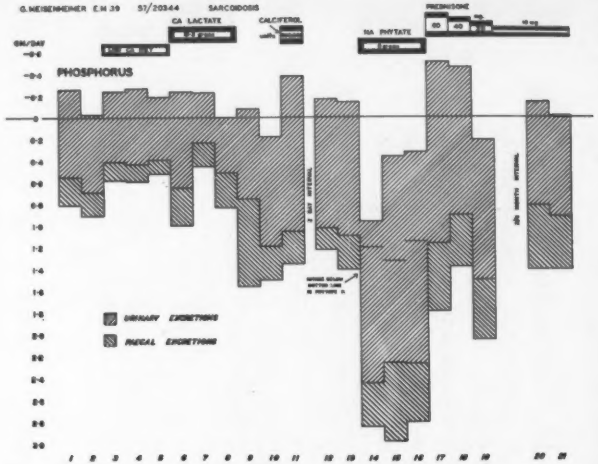
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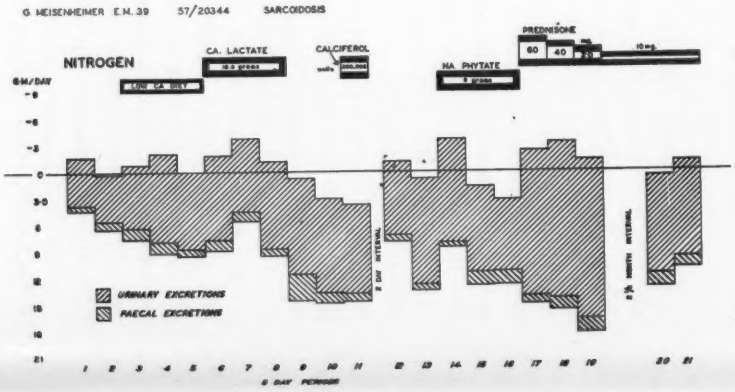
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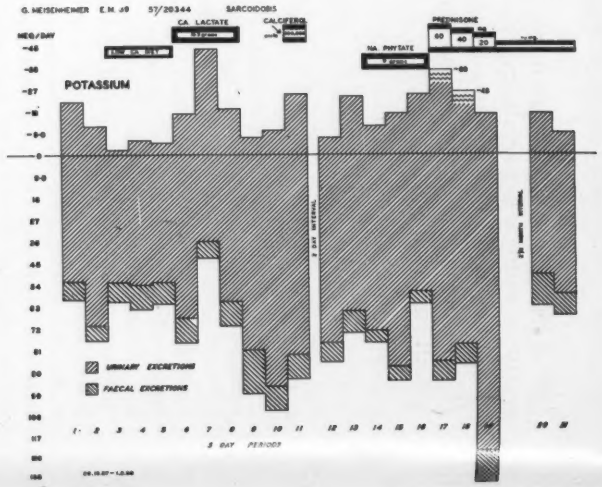
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much lower in the 2 following control periods (Nos. 12 and 13). The serum phosphorus level rose to a maximum of 5.6 mg. per 100 ml. within ten days after injection of the calciferol. The blood urea level also rose. Both slowly returned to their previous values.

Sodium phytate was given for 3 periods. It caused a reduction in urinary calcium, but only a moderate increase in fecal calcium. As with previous patients receiving phytate, a positive balance for both calcium and phosphorus was achieved.

During the first prednisone period (No. 17) urinary calcium excretion rose markedly to 800 mg. a day, but fell during the next 2 periods. Fecal calcium rose progressively during prednisone therapy. Phosphorus metabolism was not grossly altered, except for a moderate negative balance during Periods Nos. 17 and 18, which was accompanied by a similar negative potassium balance. By the fifteenth day of treatment the serum calcium level was normal (10.7 mg. per 100 ml.) for the first time.

The 2 final balance studies were carried out after two months of treatment with prednisone. The fecal calcium was normal (600 mg. per day), and the urinary calcium perhaps slightly high (200 mg. per day). The patient was in a state of nitrogen balance, but still appeared to be losing some potassium. Later, isolated 24-hour collections of urine were found to contain about 150 mg. of calcium.

Comment. Once again the fall of urinary calcium during periods of low intake was only partial, and the urinary excretion greatly exceeded the intake, even after twenty-five days. This patient appeared more sensitive than the others to increases in dietary calcium and responded by a rise in serum calcium level despite the fact that there was no great increase in absorption. His unusual sensitivity to vitamin D, however, was shown by the immediate response in serum calcium concentration. Urinary calcium excretion did not rise, possibly because of the functional renal impairment, as indicated by the rising levels of blood urea and serum phosphorus. It is interesting that no increase in intestinal absorption occurred until after calciferol had been stopped. The extra serum calcium, then, presumably came from bone, and in this instance the toxic dissolution effect of the

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FIG. 4. *Case 4.* High urinary calcium and negative balance for first 5 periods (low-calcium intake), without change in serum calcium level.

Immediate rise in serum calcium level during vitamin D therapy, preceding the fall in fecal calcium and the rise in urinary calcium. Gradual improvement of calcium metabolism during prednisone therapy, though the immediate effects included increased urinary output of all elements (see text).

vitamin on bone was evident earlier than the hypercalciuric or the absorptive effect.

Prednisone again seemed the most effective treatment. The serum calcium level was normal within fifteen days of starting this drug, and urinary calcium, after an initial rise (as may occur in normal subjects), gradually fell to about 150 mg. within three months. The initial strongly negative potassium and nitrogen balances presumably indicated an early protein catabolic effect, as may also be seen in normal subjects. Prednisone thus appeared to have 2 distinct metabolic effects; 1) a characteristically "destructive" nonspecific catabolic one, and 2) a specific "anti-sarcoid" one, with reference to normalization of calcium metabolism.

IDIOPATHIC HYPERCALCIURIA

Case 5

S.T. (No. 97442) was a white construction engineer aged 36. In the five years before we saw him he had had several attacks of renal colic. Bilateral radio-opaque renal calculi were found. The only other abnormality discovered was a urinary calcium output of 600 mg. a day. Then, and in the three years since, over 50 estimations of serum calcium and phosphorus concentration were made and all were within our normal range. The alkaline phosphatase and serum protein levels also remained normal but the blood urea level rose to 50 mg. per 100 ml. There was no radiographic evidence of bone disease. The response of phosphorus metabolism to intravenously administered calcium was normal, as was the tubular maximum reabsorption of phosphorus. Biopsy showed a normal liver. The patient worked outdoors in summer, but there was no seasonal exacerbation, nor other change in urinary calcium output.

Balance studies (Fig. 5). Nine 5-day balance periods were undertaken, all except the first one being carried out in the patient's home. Like the fourth patient (G.M.), he was a most exact and meticulous person. Periods 1, 7, and 8 were controls, with a normal daily intake of 800–1000 mg. of calcium. The findings in all 3 periods were essentially similar, although there was a mildly negative balance with a slightly lower intake in Period 8. The time intervals, as indicated on Figure 5, ensured that the studies were performed in different seasons of the year. Urinary excretion of calcium remained at about 600 mg. a day, with low fecal calcium and an increased urine/stool ratio. There was virtually no net loss of calcium from the body.

With the unusually high calcium intake of over 3.2 Gm. a day, the urinary calcium increased only slightly (by less than 200 mg.), whereas the fecal moiety became very large and the net balance was maintained as before. For Periods 4 and 6, a diet low in calcium was taken. Urinary calcium excretion was reduced, but only to 400 grams, not to normal figures. The balance consequently became grossly negative, despite a tiny fecal fraction. The same diet was taken for three months prior to the

second period (No. 6) of low-calcium intake, but virtually the same result was obtained. There was no change in serum calcium or phosphorus levels.

Sodium phytate was given, as in Case 4. This was successful in causing a partial reduction in urinary calcium and an increase in fecal calcium, but a negative balance was produced. In Period 5 a diet as high in natural phytate[1] as we could obtain was tried. However, despite some increase in fecal calcium, there was no fall in the urinary level.

Hydrocortisone was given in Period 9, but unfortunately severe dyspeptic symptoms necessitated its withdrawal. During the short time of its administration no change occurred in the balances, except perhaps for a slight increase in urinary calcium.

The phosphorus balance was unremarkable throughout. A positive balance was achieved during administration of sodium phytate, and a negative one was maintained with the low-calcium and low-phosphorus intake, even after three months.

Comment. In this case (as in the sarcoid patients) there appeared to be a sort of obligatory urinary calcium loss, which was changed comparatively little by enormous alterations in dietary calcium. With a normal intake, fecal calcium was low, *i.e.*, increased intestinal absorption had the effect of compensating for the excessive urinary loss, so that the whole body was just in balance for calcium. This state of affairs was not changed by exposure to sunlight in a South African summer, although the patient was an outdoor worker. Certainly a great net reduction in calcium intake, whether by low-calcium diet or by sodium phytate, did cause a reduction in excretion, but a considerable degree of hypercalciuria continued and the persistent negative balances indicated that a drainage of calcium and phosphorus was occurring from the bones, which must eventually lead to demineralization.

In the management of this patient, a long-continued low-calcium intake or phytate supplement would not appear to be satisfactory, because of the incomplete drop in urinary calcium so obtained and the danger of demineralization. Cortisone might have been helpful if it could have been continued for a longer period, but there is no evidence for this. The only alternative seems to be a regimen of high fluid intake, especially during the night.

Case 6

P.J. This white physician, aged 38, quite fortuitously was found to be excreting consistently some 450 mg. of calcium daily in his urine. This excretion continued un-

[1] The diet consisted largely of oat porridge (42 mg. phytate per 100 Gm.), 95–100% extraction bread (50 mg. phytate per 100 Gm.), and rye vita (300 mg. phytate per 100 Gm.).

changed for the two years of observation to date, and bore no relation to the season, despite considerable exposure to the summer sun. He remained perfectly well, with no renal calculi and no abnormality in the serum levels of calcium, phosphorus, phosphatase, protein or urea. His intake was never abnormally high in vitamin D or calcium, the average daily ingestion of milk being under half a pint. As discussed previously, it seems reasonable to consider this condition as "healthy idiopathic hypercalciuria."

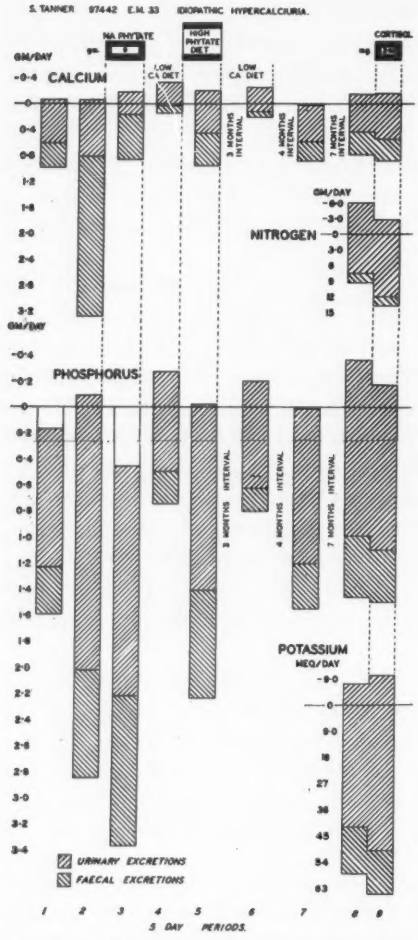


FIG. 5. *Case 5.* Hypercalciuria is only slightly altered by enormous changes in the calcium intake. Negative balance occurred only during period of low intake. No seasonal variation (see text).

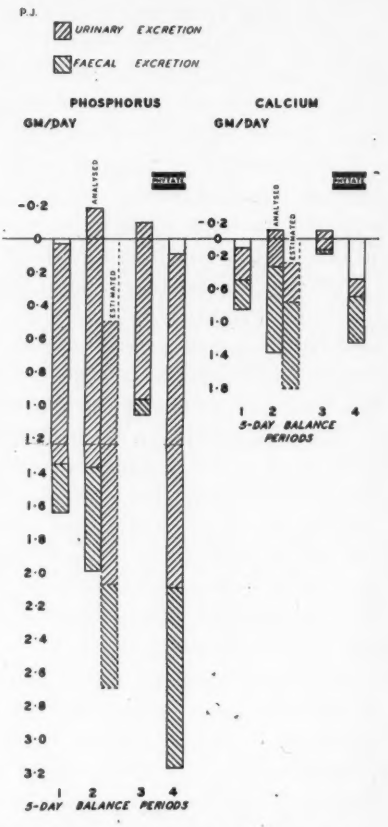


FIG. 6. *Case 6.* A "healthy hypercalciuric" subject. Findings similar to those in Figure 5. Phytate appeared to cause some reduction in urinary calcium without producing a negative balance (see text). Note high urine/stool calcium ratio (6:1) during period of low-calcium intake and compare with data in Figure 7. (Unexplained error in analysis in Period 2; estimated figures believed more accurate.)

Balance studies (Fig. 6). Four periods were completed in the subject's own home, while a perfectly normal life was being pursued. With a normal diet containing some 800 mg. of calcium per day, he was in a state of calcium and phosphorus balance. The urine/stool phosphorus ratio was normal, but there was (as in Case 5) a high absorption of calcium from the gut (*i.e.*, low fecal excretion).

In the second period there was unfortunately some error in the analyses, since more calcium was taken as milk than is shown for the whole intake as analysed. The balance as estimated from food tables and from a separate analysis of milk in our own laboratory, is therefore to be considered to be more correct (*i.e.*, the second columns under our Period 2 in Figure 6). Urinary calcium excretion showed virtually no increase during the period of high-calcium diet.

The low-calcium intake (Period 3) resulted in considerable reduction of urinary calcium excretion, but only to 250 mg. a day. Thus a negative balance was produced, despite the negligible amount of fecal calcium.

Sodium phytate caused, as expected, increased excretion of both fecal and urinary phosphorus. The effect on calcium balance, however, was surprising. There was little change in fecal calcium but considerable reduction in urinary calcium—and consequently a positive balance.

Comment. The studies on P. J. showed virtually the same phenomena, though in lesser degrees, as those on S. T. (Case 5). The high urinary calcium occurred in association with low fecal calcium, so that there was no net loss to the body when a normal diet was taken. With a low-calcium intake, however, the urinary calcium remained at an abnormally high level, and was only slightly changed by an intake of calcium that was double the normal. This again suggests a basic "obligatory" high renal output of calcium. It is noteworthy that this very high urine/stool calcium ratio of 6:1 during the period of low-calcium intake was the exact opposite of what we have found in normal subjects with a urinary calcium output of the usual dimensions (*e.g.*, Subject A.M., Fig. 7). In these normal persons, fecal calcium excretion remains much higher than the meager urinary excretion.

ACROMEGALY WITH HYPERCALCIURIA

Case 7

Mr. D, aged 47. In acromegaly, osteoporosis may develop with, at some stage, a raised urinary excretion of calcium. This man with acromegaly had a continued high level of urinary calcium excretion (as high as 800–1000 mg. when untreated) without symptomatic or radiologic evidence of bone disease. Unfortunately he had been treated with testosterone and had just started a course of x-irradiation to the pituitary before the only 3 balance periods we were able to perform. Consequently, his urinary calcium output had been reduced to a mere 450 mg. a day.

Typical acromegalic symptoms had been present for fourteen years. The patient also had severe diabetes and took 80 units of insulin daily. He complained of thirst and polyuria, increased coarsening of the features and some weakness and lethargy. His eyes were not affected. Roentgenograms of the skull, hands, spine and long bones were entirely characteristic of acromegaly and the serum phosphorus level was about 4.5 mg. per 100 ml. on several occasions.

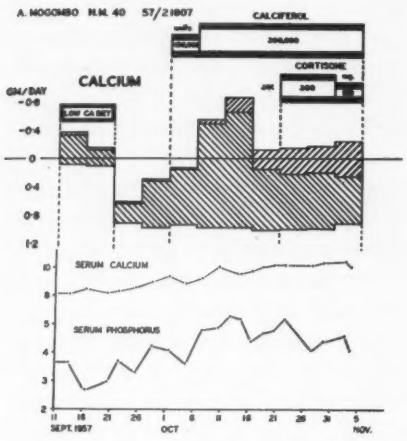


FIG. 7. *Normal subject* (Bantu male). Note low urine/stool calcium ratio (1:6) during low-calcium intake, in contrast to the data in Figure 6. Note also the rise in urinary calcium during calciferol therapy, along with a *rise* in stool calcium. The serum calcium also rose, but remained within the normal range. (The normal serum calcium level in the Bantu may be as low as 8 mg. per 100 ml.)

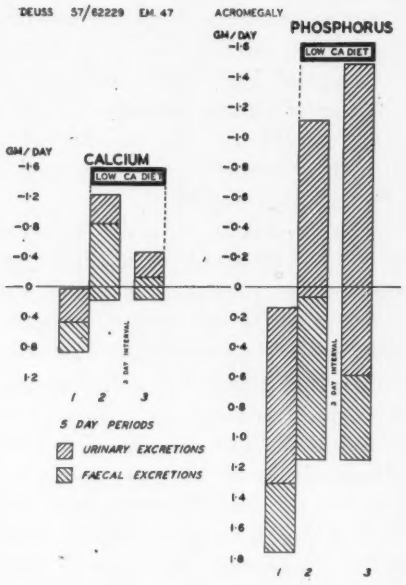


FIG. 8. *Case 7.* When calcium intake was normal, there was a high urinary output of calcium but a low output of fecal calcium, without a negative balance. Note little change in urinary calcium during low intake (see text).

Balance studies (Fig. 8). With the normal dietary intake of 800 mg. of calcium, hypercalciuria was present. As in sarcoid and idiopathic hypercalciuria, however, increased intestinal absorption prevented the calcium balance from becoming negative. Also as in the other conditions, a restricted calcium intake immediately produced a negative balance with little change in urinary calcium.

Once again, therefore, it would appear that hypercalciuria during a period of normal intake could be compensated for by increased intestinal absorption.[2] This would explain why demineralization of bones did not

[2] The difficulty about this argument is the very high fecal calcium in the first period, with a low-calcium intake. This suggests that there may have been errors in stool collections.

occur in this case, despite persistent severe hypercalciuria.

In this connection it is interesting that the effect of human growth hormone on the calcium metabolism of the adult (17) is to produce an increased urinary excretion, while maintaining the subject in a state of calcium balance. In other words, growth hormone induces over-absorption from the intestine with a high urinary loss, as we observed in our case of acromegaly.

THE HYPERCALCIURIA OF HYPERPARATHYROIDISM

The high urinary calcium excretion of hyperparathyroidism follows the rise in the level of serum calcium, which is one of the primary effects of the parathyroid hormone. Balance studies conducted by the Albright group (4, 18, 19) show that there may be a very low fecal excretion of calcium, and that a reasonably high calcium intake may indeed prevent demineralization. Parathyroid hormone is not known to have any direct effect on calcium absorption, so here again is a strong suggestion that the hypercalciuria leads to increased intestinal absorption which in effect compensates the body for the excessive urinary loss of calcium.

THE HYPERCALCIURIA PRODUCED BY VITAMIN D

There is ample evidence that vitamin D has several distinct effects on calcium metabolism:

1. Increased intestinal absorption (9).
2. Increased urinary output of calcium (12, 20, 21, 22).
3. Elevated serum calcium level.
4. Demineralization of bone (prolonged effect of large doses).

It seems likely that the first 3 actions may be independent of each other in that: 1) a raised serum calcium level can occur before or without increased absorption (*e.g.*, Fig. 4); 2) a raised urinary excretion of calcium can occur without a change in fecal or serum calcium, and may often be seen as the first effect of vitamin D (*e.g.*, Figs. 7 and 9); and 3) a raised serum calcium level can occur without any change in urinary calcium (*e.g.*, Case 4 in Period 11). Dent (21) reported a large *fall* in urinary calcium in a case of osteomalacia under treatment, while the serum calcium level rose.

The hypercalciuric action of vitamin D in general has been rather neglected. It seems to be an early effect in normal subjects, in rickets of the ordinary and "resistant" varieties (Fig. 9), and in hypoparathyroidism. In view of this, one is led to wonder whether the increased urinary calcium may not be the stimulus to increased intestinal absorption, as apparently happens in other circumstances discussed here. Can part of the action of vitamin D be expressed as:

raised urinary calcium (presumably diminished renal tubular reabsorption)
→ raised intestinal absorption?

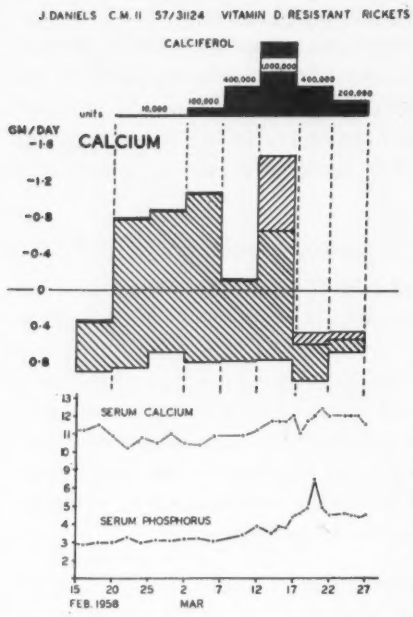


FIG. 9. Pronounced rise in urinary output of calcium during administration of 1 million units of calciferol, without any reduction in fecal calcium until the next balance period. The maximum rise in serum calcium concentration occurred when urinary calcium excretion has diminished.

DISCUSSION

Hypercalciuria in the idiopathic group

In our 2 subjects it would appear that the urinary calcium remained higher than normal, regardless of the intake. Albright *et al.* (23) and Pyrah (24) also reported metabolic balance studies in patients in whom a low-calcium intake resulted in a negative balance despite very low fecal excretion. It does not seem likely that the primary defect here can be intestinal over-absorption, since if this were so, a reduced intake should rapidly prevent hypercalciuria. There is no evidence that vitamin D plays any part in this syndrome, and hypercalcemia does not appear. It would seem, then, that the over-absorption of calcium from the gut can be looked upon as a compensatory mechanism for the excessive urinary loss. When the subject is receiving a normal diet, compensation is complete inasmuch as there is no depletion of body calcium stores. When the intake of calcium is low, the gut cannot compensate because it has nothing to work on.

This disorder may be represented as follows:

? Decreased tubular reabsorption of calcium
↓
Increased urinary calcium
↓
Increased intestinal absorption.

The hypercalciuria of sarcoidosis

The changes in calcium metabolism found in sarcoid are: 1) over-absorption from the intestine, 2) hypercalciuria of a greater degree than can be explained by over-absorption, 3) hypercalcemia (usually), 4) hypersensitivity to vitamin D in *some cases*, especially with relation to the serum calcium, and 5) repression of these changes during treatment with cortisone, the reduction in serum calcium concentration often occurring well before the reduction in urinary calcium excretion (25, 26) and without

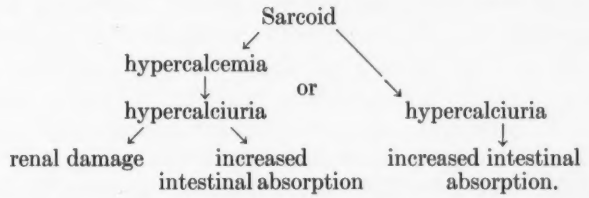
changes in fecal calcium. This is important in further strengthening the suggestion that in sarcoidosis (as in vitamin D activity) the serum calcium level may change independently of absorption and of urinary excretion.

Because of the over-absorption, generalized demineralization of bone does not occur, since in none of the patients was there a negative balance with a normal intake of calcium. However, the following progression of events:

increased intestinal absorption of calcium
↓
raised serum calcium level
↓
hypercalciuria
↓
renal damage

cannot explain 1) subjects with hypercalciuria without hypercalcemia, nor 2) how urinary calcium output can so greatly exceed the total intake during periods of low-calcium diet. It is difficult to avoid the conclusion that there is also a primary renal defect in calcium excretion (presumably a diminution in tubular reabsorption).[3] In Case 1, since urinary calcium was unchanged by a very low calcium intake, the intestinal mechanism appeared to play no part at all in the high urinary level. In the other cases also, hypercalciuria was independent (at least to some extent) of both the serum calcium level and intestinal absorption.

It is possible, however, that there is a completely different progression of events, *i.e.*, the hypercalciuria may lead to compensatory intestinal overabsorption. This would make hypercalcemia the primary disorder in the cases in which it occurs. The relationships may be represented as follows:



There is a good deal of evidence that this sequence of events may occur in other conditions, as discussed in this report.

It is plain that hypersensitivity to vitamin D *could* explain all these phenomena, especially since vitamin D intoxication responds just as readily to cortisone as do the calcium upsets in sarcoidosis (25, 27). The mechanisms might then be:

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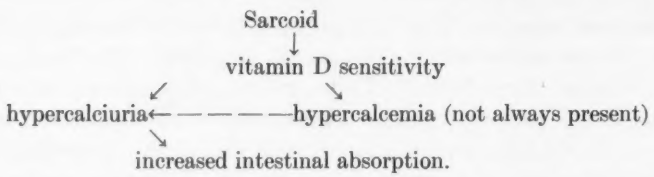
[3] It is possible that there might be a change in the character of the serum calcium to account for this condition, rather than an actual renal dysfunction, but there is no evidence in favor of this hypothesis.



A further feature in favor of this hypothesis is the very small change in urinary calcium which is produced by a large increase in ingested calcium. If the primary condition were an excessive absorption from the bowel, surely this absorptive capacity would be likely to show itself with the larger intake also? If, however, it were a compensatory mechanism, then it would be natural for it to be self-limited.

One difficulty in the hypothesis that vitamin D sensitivity accounts for the calcium disturbances in sarcoidosis is that not all patients appear to be sensitive to fairly large doses (*e.g.*, Cases 2 and 3). The great and rapidly occurring sensitivity in some other subjects (*e.g.*, Case 4) suggest that there must be at least 2 groups of sarcoid patients with calcium disturbances—those who are sensitive to vitamin D, and those who are not. Fortunately, the latter group also appear to respond well to adrenocortical steroids.

Concept of intestinal compensation

We have suggested that, as a bodily principle, when there is excessive loss of calcium in the urine, the intestine will compensate as far as possible by an increased absorption of calcium. This relationship will not *always* hold (*e.g.*, not in immobilization), but if it be looked for, perhaps it may be found with surprising frequency. This chain of events must normally be occurring all the time, since different normal adults may have consistently different urinary calcium outputs, ranging from 50 mg. in some cases to 400 mg. per day in others. These great individual variations of urinary calcium in normal adults, the relative constancy of urinary calcium excretion in each subject over long periods under normal conditions, and the extremely small changes produced in urinary calcium by great changes in calcium intake have been fully attested on a large scale by Knapp (28), Nicolaysen *et al.* (29), and Hodgkinson and Pyrah (30). Yet, whatever the natural level of urinary calcium may be, each normal adult maintains a net bodily balance for calcium when considered over long periods. Thus his intestinal absorption must be naturally compensating and attuning itself to the level of urinary loss. The absorptive calcium "barrier" is therefore controlled by the urinary output. The necessary corollary, that of some form of humoral control of calcium absorption, has already been postulated by Nicolaysen (31) to explain the high absorption in growing children and after fractures. A further corollary might be postulated, namely, that when intestinal compensation for urinary calcium loss does

not occur, a net negative balance persists and eventually decalcification appears—as in the osteoporosis associated with immobilization, or even in ordinary osteoporosis.

Acknowledgments

We should like to thank our patients for voluntarily submitting themselves to these balance studies, Miss M. Lloyd and Miss E. Phillips for their technical assistance, and Staff Nurse Schoonraad for his great help in the metabolic ward.

Professor C. E. Dent, Professor J. F. Brock and Dr. R. Hoffenberg have made very valuable criticisms of the manuscript.

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Addendum

Since writing this paper we have seen an article on Idiopathic Hypercalciuria by Henneman, P. H.; Benedict, P. H.; Forbes, A. P.; and Dudley, H. R.: *New England J. Med.* **259**: 802, 1958. Their findings with metabolic balance studies corroborate ours as follows: 1) fecal calcium is low in this condition; 2) the hypercalciuria is maintained despite a low-calcium intake; 3) the patients are in a state of negative calcium balance during the period of low intake; 4) this net loss of calcium is increased when phytate is administered; and 5) urinary calcium is increased by cortisone therapy.

The authors suggest, as we do, that "increased calcium absorption would explain the absence of osteomalacia in these patients despite years of urinary calcium loss" (sic). They consider, but reject, the hypothesis that a factor (*e.g.*, hypercalciuria) which tends to deplete the body of calcium may lead to increased calcium absorption. They supply no alternative suggestion.

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THE ACUTE EFFECTS OF PARATHYROID EXTRACT IN STATES OF EDEMA, DIMINISHED RENAL FUNCTION AND PARATHYROID DISEASE*†

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ABSTRACT

In a study of 42 patients it was found that parathyroid extract inhibits the tubular reabsorption of phosphorus. When it is injected intravenously, the effect begins immediately and reaches a peak in an hour. The extract also transiently increases: 1) renal plasma flow, apparently due to a generalized vasodilatation which is not related to the phosphorus effect, and 2) sodium and water excretion in edematous subjects. In a group of patients with diminished filtration rates the control phosphorus reabsorption was decreased. In these cases, parathyroid extract induced the usual decrease in phosphorus reabsorption. In a patient with hyperparathyroidism, although the control phosphorus reabsorption was low, parathyroid extract did not decrease it. The difference in response was helpful in distinguishing these 2 conditions.

IT HAS long been known that injection of parathyroid extract (PTE) in man induces a prompt increase in urinary phosphorus (1). Transient hemodynamic changes after the administration of the extract have also been described. The latter observations have led some investigators to claim that the phosphaturia is secondary to an increase in the glomerular filtration rate, whereas others assert that the extract inhibits renal tubular reabsorption of phosphorus (2–4). Recently it has been shown that the extract increases renal blood flow and urine flow promptly in patients with oliguria induced by intravascular hemolysis (5). In view of these observations, we investigated the immediate hemodynamic and electrolyte (phosphorus, sodium and potassium) effects of the extract in normal subjects, in patients with edema and oliguria, and in patients with diminished renal plasma flow and glomerular filtration rate.

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MATERIALS AND METHODS

Forty-two subjects were studied. Of these, 5 were normal or convalescent, and 37 were patients selected from the wards of Bellevue Hospital.

The studies were performed in the morning between 8 and 10 a.m. in order to avoid the normal diurnal variation in phosphorus excretion (6). The subjects fasted overnight but were allowed water. For measurement of glomerular filtration rate (GFR) and effective renal plasma flow (RPF), a solution of p-aminohippurate and insulin was infused intravenously by means of a constant-rate infusion pump. GFR was measured as the clearance of inulin, and RPF as the clearance of PAH. Urines were collected through an indwelling catheter, with the aid of water and air washouts. When parathyroid extract (PTE)[1] or Mercuhydrin was given, three 15-minute collections served as control specimens. The PTE (200 units) or Mercuhydrin (2 ml.) was injected intravenously over a period of four to five minutes, and the study was continued for 4 additional periods of fifteen to twenty minutes each.

Inulin and p-aminohippurate values were determined by the methods of Schreiner (7) and of Smith *et al.* (8), respectively, inorganic phosphorus by the Fiske and Subba-Row method (9), and sodium and potassium by means of a flame photometer. Since many of the patients had edema and ascites, the values for filtration rate and renal plasma flow were not corrected for surface area.

Phosphorus reabsorption (TRP) was calculated as the percentage of the filtered phosphorus reabsorbed, using the formula,

$$\frac{P_{po_4} \times GFR - U_{po_4}V}{P_{po_4} \times GFR} \times 100.$$

RESULTS

Effect of parathyroid extract on RPF, GFR and electrolyte excretion in subjects without edema

Eleven subjects received injections of PTE. This group included 5 normal persons and 6 patients without evidence of renal or parathyroid disease. Detailed data on 4 cases are given in Table 1. The RPF increased consistently within fifteen to twenty minutes and usually returned to the control level within sixty to ninety minutes. The peak increase varied from 14 to 94 per cent and was not related to the initial RPF or to the systemic blood pressure. Both diastolic and systolic blood pressures fell within five minutes. This decrease, on the average, was 15 mm. Hg and lasted about twenty minutes. A reaction to the injection was seen in 2 patients; one had a generalized flush and the other, an asthmatic, had a brief attack of coughing.

The effect on GFR was variable. In 13 patients the GFR increased temporarily by 7 to 20 per cent, in 9 it decreased by 6 to 26 per cent, and in the others no change occurred. The alterations observed were not related to the initial levels.

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[1] We are indebted to Eli Lilly and Company for supplying the parathyroid extract. Three lots were used, all of which were active.

Phosphorus excretion increased promptly in every patient regardless of the change in filtration rate, indicating a specific effect on phosphorus reabsorption (Table 1, Subjects NE, MU and BU). The peak was reached in the first sixty minutes and lasted for several hours. The degree of increase varied considerably but appeared to be inversely related to the initial level. TRP decreased immediately and reached its lowest level within sixty minutes. One exception was observed; a patient with diabetes

TABLE 1. EFFECT OF PARATHYROID EXTRACT IN SUBJECTS WITHOUT EDEMA

Subject	Time (mins.)	GFR (ml./min.)	RPF (ml./min.)	Urine flow (ml./min.)	Phosphorus		Phosphorus reabsorbed		Na excreted (mEq./min.)	K excreted (mEq./min.)
					Plasma (mg./100 ml.)	Excreted (mg./min.)	(mg./min.)	(% filt. load)		
NE, convalescent from stroke	0*	102	328	7.1	4.40	0.871	3.617	80	0.390	
	0†–8	99	368	11.5	4.30	1.104	3.153	74	1.740	
	8–25	85	375	7.9	—	1.375	2.280	62	0.782	
	25–39	79	339	4.4	4.35	1.113	2.324	68	0.432	
	39–56	90	333	4.3	4.35	1.402	2.513	64	0.336	
	56–70	94	310	5.3	—	1.320	2.769	68	0.387	
MU, after hypophysectomy for chromophobe tumor	0*	112	541	1.4	4.40	0.705	4.233	87	0.036	0.098
	0†–13	103	705	3.9	—	0.936	3.596	79	0.570	0.245
	13–27	92	694	3.7	4.40	1.310	2.738	68	0.446	0.412
	27–44	91	568	2.1	4.25	1.275	2.593	68	0.264	0.324
HO, idiopathic osteoporosis	0*	74	356	4.6	2.85	0.290	1.819	87	0.089	0.022
	0†–15	80	416	3.1	—	0.390	1.890	83	0.192	0.034
	15–34	82	494	3.0	2.85	0.491	1.846	79	0.275	0.044
	34–55	75	514	1.7	—	0.583	1.555	73	0.164	0.029
	55–76	69	480	1.2	2.90	0.600	1.400	70	0.149	0.023
BU, Paget's disease	0*	108	393	0.7	3.65	0.495	3.445	88	0.124	0.055
	0†–11	112	522	1.2	—	0.655	3.425	84	0.195	0.085
	11–28	131	514	1.8	—	0.891	3.879	81	0.258	0.130
	28–42	91	373	0.9	3.65	0.770	2.550	77	0.172	0.074
	42–57	87	373	0.7	—	0.771	2.410	76	0.129	0.050

* In this and all subsequent tables, the control values are the average for three 15-minute periods.
† A dose of 200 units of PTE was injected at time 0.

insipidus and adrenal insufficiency who was being treated with cortisone and salt did not show the fall in TRP until the second hour after injection.

Sodium excretion increased promptly and usually had returned to the initial level by the end of ninety minutes, paralleling the change in RPF. Potassium excretion also increased transiently, but not always at the same time as sodium excretion (Table 1, Subject MU).

Urine flow increased immediately in most cases, and usually returned to the initial level by the end of sixty to ninety minutes. In an occasional subject who had reached the end of a water diuresis period at the time of the injection, no increase in urine flow occurred (Table 1, Subject HO).

Effect of parathyroid extract in patients with edema (repeated studies)

Fifteen patients with edema were studied—5 with congestive heart failure and 10 with cirrhosis of the liver and ascites. The results obtained in 4 typical cases are shown in Table 2. In all 15 patients the response to PTE was virtually the same as in the group without edema. RPF, urine flow, and sodium and potassium excretion increased in the first thirty minutes, and returned to the initial levels at the end of ninety minutes. Phosphorus excretion increased promptly and remained elevated.

TABLE 2. EFFECT OF PARATHYROID EXTRACT IN 4 PATIENTS WITH EDEMA

Subject	Time (mins.)	GFR (ml./ min.)	RPF (ml./ min.)	Urine flow (ml./ min.)	Phosphorus		Phosphorus reabsorbed (% filt. load)	Na excreted (mEq./ min.)	K excreted (mEq./ min.)
					Plasma (mg./ 100 ml.)	Excreted (mg./ min.)			
KE, cirrhosis of the liver with ascites	0	72	193	2.0	3.40	0.695	85	0.005	0.090
	0*–11	63	185	1.9	3.35	0.649	69	0.005	0.103
	11–25	85	282	4.4	3.25	1.280	54	0.017	0.290
	25–39	83	262	4.1	3.40	1.420	50	0.013	0.272
LU, cirrhosis of the liver with ascites and pyelonephritis	0	23	179	1.1	5.10	0.586	51	0.003	0.064
	0*–13	16	194	0.9	—	0.435	47	0.004	0.051
	13–31	32	274	1.8	4.85	0.936	41	0.008	0.118
	31–51	27	189	1.9	—	0.932	29	0.005	0.095
	51–64	26	191	1.9	4.30	1.037	7	0.006	0.090
PE, congestive heart failure, nephritis	0	20	100	1.0	3.95	0.294	63	0.070	—
	0*–16	22	102	1.2	4.10	0.333	63	0.095	—
	16–28	17	119	1.3	—	0.401	40	0.092	—
	28–41	19	114	1.2	3.75	0.407	43	0.074	—
	41–59	25	139	1.3	—	0.565	40	0.080	—
	59–71	22	121	1.2	—	0.488	41	0.077	—
MA, congestive heart failure	0	69	205	1.0	3.25	0.130	94	0.043	0.037
	0*–13	61	224	0.7	—	0.113	95	0.032	0.044
	13–26	53	263	1.5	3.25	0.275	84	0.098	0.114
	26–42	59	204	1.2	—	0.278	85	0.063	0.101

* A dose of 200 units of PTE was injected at time 0.

The extract was injected twice in 5 patients and three times in 1 patient at intervals of from one to twelve weeks. In 4 of the 6 subjects the decrease in TRP was the same as initially, when PTE was injected the second time. The changes in sodium and potassium excretion and in GFR and RPF showed a greater variation in the second study.

In no case did PTE induce a significant loss of weight in the twenty-four hours following its administration.

Control experiments with Mercuhydrin

The acute effect of Mercuhydrin on phosphorus reabsorption was studied in 8 patients with edema. Phosphorus reabsorption did not decrease significantly (average fall, 2 per cent; range, 0 to 7 per cent) (Table

3 and Fig. 3) in the forty-five minutes following injection of Mercuhydrin. When PTE was then injected, the TRP decreased promptly.[2]

Tubular reabsorption of phosphorus (TRP)

The level of TRP in the fasting state has been reported to be 78 to 90 per cent of the filtered load in normal subjects (10). In our group of patients with filtration rates above 49 ml. per minute, TRP was 80 to 97 per cent. In 3 patients with filtration rates above 49 ml. per minute, the TRP was 77 per cent. One of these 3 patients had idiopathic hypoproteinemia treated with triiodothyronine; 1 had cirrhosis of the liver and ascites treated with Diamox, and 1 had adrenal insufficiency treated with cortisone.

In patients with filtration rates below 49 ml. per minute, the TRP was diminished.[3] Eleven studies were carried out on 8 patients with filtration rates of from 18 to 48 ml. per minute. In these cases the TRP ranged from 46 to 75 per cent and varied with the filtration rate (Fig. 1). The group included 4 patients with cirrhosis of the liver (2 of whom had nephritis), 1 with congestive heart failure, nephritis and diabetes mellitus, 1 with congestive heart failure and recurrent renal calculi, 1 with metastatic carcinoma of the breast, and 1 with untreated myxedema. The plasma inorganic phosphorus level was elevated in 2 of these patients (5.20 and 5.25 mg. per 100 ml.); it was between 3.25 and 4.85 mg. per 100 ml. in the other 6.

The RPF did not appear to be significantly related to the TRP (Fig. 2). Half of the patients with an RPF less than 200 ml. per minute had a normal TRP.

Effect of parathyroid extract on TRP in patients with a GFR less than 49 ml. per minute

PTE promptly inhibited phosphorus reabsorption in all patients (Fig. 3). In those with filtration rates greater than 49 ml. per minute the decrease in TRP averaged 16 per cent, with a range of 8 to 40 per cent. In 8 studies on 7 patients with filtration rates less than 49 ml. per minute the average decrease in TRP was 36 per cent, with a range of 16 to 52 per cent. Detailed

[2] Sodium excretion increased promptly after injection of PTE. This may have been in large part due to the Mercuhydrin, which had attained its maximal natriuretic effect at that time. However, patients who received injections of both substances lost more weight than when treated with Mercuhydrin alone—suggesting that PTE synergized the diuretic effect of Mercuhydrin.

[3] Two exceptions have recently been found. One patient (Table 5, Subject SA) was a poorly nourished woman with cirrhosis of the liver, massive ascites and pyelonephritis in whom the filtration rate was 13 ml. per minute and the TRP 85 per cent. The other was a woman with postpartum panhypopituitarism, in whom the filtration rate was 36 ml. per minute and the TRP 80 per cent.

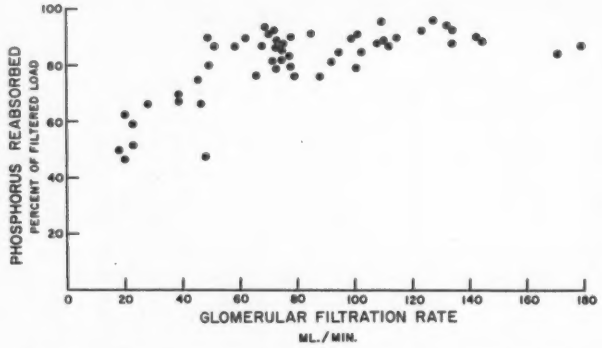

FIG. 1. Phosphorus reabsorption in relation to glomerular filtration rate. Each circle is the average of three 15-minute periods.

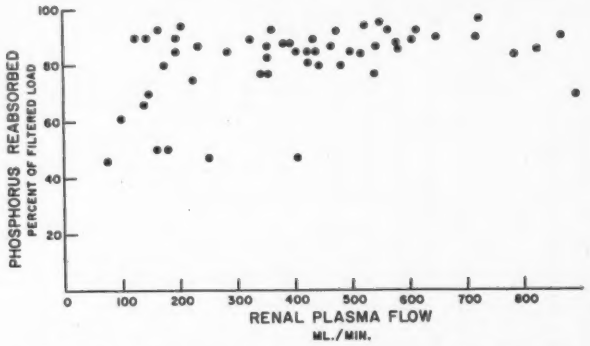

FIG. 2. Phosphorus reabsorption in relation to renal plasma flow. Each circle is the average of three 15-minute periods.

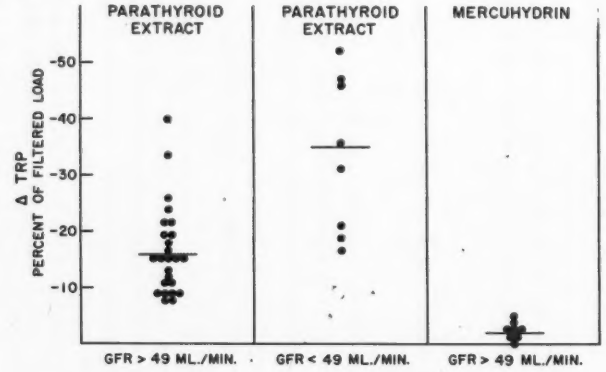

FIG. 3. The effect of parathyroid extract on phosphorus reabsorption in patients with filtration rates greater and less than 49 ml. per minute. Each circle represents the decrease based on the average of the two or three lowest values in the hour after injection. The horizontal line is the average decrease for each group.

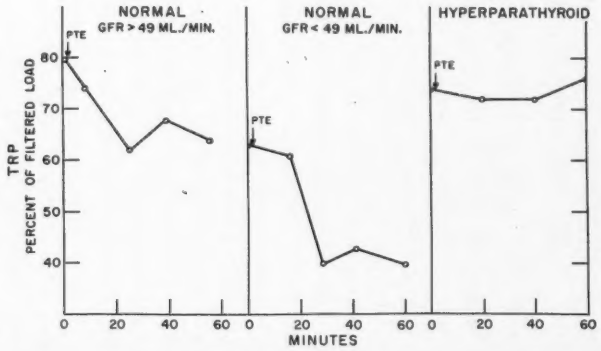

FIG. 4. Comparison of the effect of parathyroid extract in a subject with a normal filtration rate, with a filtration rate less than 49 ml. per minute, and in the patient with hyperparathyroidism.

TABLE 3. EFFECT OF MERCUHYDRIN AND PARATHYROID EXTRACT IN 3 PATIENTS WITH EDEMA

Subject	Time (mins.)	GFR (ml./min.)	RPF (ml./min.)	Urine flow (ml./min.)	Phosphorus		Phosphorus reabsorbed (% filt. load)	Na excreted (mEq./min.)	K excreted (mEq./min.)
					Plasma (mg./100 ml.)	Excreted (mg./min.)			
MA, cirrhosis of the liver with ascites, treated with Diamox	0	95	428	2.6	3.15	0.453	85	0.145	0.025
	0*–10	105	456	3.4	3.20	0.544	84	0.245	0.029
	10–23	98	431	2.9	—	0.597	81	0.246	0.025
	23–36	93	455	2.3	2.95	0.401	85	0.220	0.018
	36†–51	88	577	3.2	—	0.643	75	0.299	0.024
	51–62	103	775	4.7	—	1.196	61	0.536	0.045
	62–74	91	672	3.1	2.80	1.095	57	0.372	0.031
	74–85	94	682	3.3	—	1.171	56	0.391	0.025
BR, cirrhosis of the liver with ascites	0	92	481	0.8	3.80	0.634	82	0.103	0.051
	0*–13	87	426	1.0	3.80	0.589	82	0.148	0.054
	13–27	100	406	1.1	—	0.726	81	0.172	0.047
	27–37	77	386	1.2	3.70	0.630	78	0.184	0.035
	37‡–51	76	515	1.6	—	0.760	73	0.246	0.045
	51–62	94	787	2.6	—	1.247	64	0.344	0.080
	62–77	80	569	2.8	3.75	1.132	62	0.373	0.077
BU, cirrhosis of the liver with ascites	0	74	463	0.7	2.60	0.257	86	0.004	0.046
	0*–15	79	548	0.8	—	0.303	85	0.008	0.055
	15–30	79	497	0.5	2.50	0.336	83	0.009	0.047
	30–42	74	514	0.6	—	0.371	82	0.009	0.044
	42§–59	82	598	0.9	2.50	0.314	85	0.045	0.060
	59–73	66	587	1.7	—	0.506	69	0.129	0.094
	73–87	79	522	1.1	2.55	0.675	66	0.067	0.063

* A dose of 2 ml. of Mercuhydrin was injected intravenously.
† A dose of 200 units of PTE was injected at 38 minutes.
‡ A dose of 200 units of PTE was injected at 39 mintues.
§ A dose of 200 units of PTE was injected at 42 minutes.

data on 4 of the latter group are given in Table 4, and data on 2 others (Subjects LU and PE) appear in Table 2. In Subject HA, administration of PTE completely inhibited reabsorption, so that all the filtered phosphorus was excreted. In all of these patients the effect on TRP was independent of the change in filtration rate.

Effect of parathyroid extract on TRP in 2 patients with parathyroid disease

One patient with pseudohypoparathyroidism was studied on two occasions (Table 5). In neither instance did PTE significantly inhibit reabsorption of phosphorus, though excretion of phosphorus increased transiently along with an increase in GFR. The RPF increased 40 per cent on both occasions.

A 63-year-old woman was studied before and after removal of a functioning parathyroid tumor (Table 5). Control studies showed a TRP of 74 per cent and a GFR of 48 ml. per minute. In view of the low GFR it was uncertain whether the low TRP was associated with the renal impairment

TABLE 4. EFFECT OF PARATHYROID EXTRACT IN 4 PATIENTS WITH FILTRATION RATES LESS THAN 49 ML. PER MINUTE

Subject	Time (mins.)	GFR (ml./ min.)	RPF (ml./ min.)	Urine flow (ml./ min.)	Phosphorus		Phosphorus reabsorbed (% filt. load)	Na excreted (mEq./ min.)	K excreted (mEq./ min.)
					Plasma (mg./ 100 ml.)	Excreted (mg./ min.)			
RO, metastatic carcinoma of the breast	0	39	145	0.7	3.60	0.424	70	0.031	—
	0*–15	35	185	1.0	—	0.479	62	0.046	—
	15–31	36	190	1.3	3.30	0.608	49	0.061	—
	31–41	34	210	1.3	—	0.732	35	0.059	—
	41–69	31	173	1.9	3.00	0.783	16	0.038	—
HA, cirrhosis of the liver	0	48	406	4.1	3.25	0.890	47	0.121	0.065
	0*–11	31	404	3.5	—	1.180†	0†	0.146	0.070
	11–24	55	607	3.9	2.90	1.710	0	0.135	0.096
	24–34	51	542	3.3	—	1.680	0	0.116	0.084
	34–47	51	457	3.0	2.75	1.610	0	0.112	0.082
SA, chronic pyelonephritis, cirrhosis of the liver	0	13	293	0.3	4.85	0.090	85	0.002	0.018
	0*–21	25	383	0.7	—	0.312	74	0.004	0.043
	21–40	19	274	0.7	4.85	0.503	45	0.003	0.045
	40–59	23	242	0.6	—	0.518	53	0.003	0.039
	59–76	21	223	0.6	—	0.550	46	0.006	0.034
	76–94	22	240	0.6	4.85	0.595	44	0.005	0.035
CU, recurrent renal calculi, congestive failure	0	20	76	1.0	4.40	0.471	46	—	—
	0*–18	22	103	1.3	4.10	0.549	33	—	—
	18–42	21	102	1.8	—	0.601	30	—	—
	42–59	19	83	1.3	4.20	0.562	30	—	—
	59–79	18	71	0.9	—	0.513	32	—	—

* A dose of 200 units of PTE was injected at time 0.

† In this and subsequent periods, phosphorus excretion exceeded the filtered load by 0.188 to 0.221 mg. per minute.

TABLE 5. EFFECT OF PARATHYROID EXTRACT IN PSEUDOHYPOPARATHYROIDISM AND IN HYPERPARATHYROIDISM

Subject	Time (mins.)	GFR (ml./ min.)	RPF (ml./ min.)	Urine flow (ml./ min.)	Phosphorus		Phosphorus reabsorbed (% filt. load)	Na excreted (mEq./ min.)	K excreted (mEq./ min.)
					Plasma (mg./ 100 ml.)	Excreted (mg./ min.)			
Pseudohypoparathyroidism	0	99	435	3.7	5.55	0.546	90	0.145	0.038
	0*–8	87	450	2.6	5.10	0.576	—	0.206	0.033
	8–24	99	614	2.6	—	0.627	88	0.336	0.059
	24–40	113	538	2.0	5.00	0.715	87	0.261	0.037
	40–53	111	445	1.3	—	0.531	90	0.197	0.025
Hyperparathyroidism† Preop.	0	48	370	2.7	2.60	0.309	74	0.118	0.040
	0*–20	68	486	7.8	—	0.490	72	0.475	0.124
	20–40	60	396	5.2	2.50	0.417	72	0.417	0.097
	40–59	46	504	2.5	—	0.346	76	0.222	0.056
	59–75	57	537	1.6	2.45	0.332	76	0.153	0.043
Postop.	0	66	407	1.6	4.20	0.514	81	0.102	0.067
	0*–18	73	508	3.2	—	0.642	79	0.144	0.126
	18–37	67	482	4.2	—	0.726	73	0.148	0.188
	37–58	72	452	2.0	3.60	0.653	75	0.111	0.113
	58–78	72	455	1.3	—	0.570	78	0.093	0.057

* A dose of 200 units of PTE was injected at time 0.

† This patient had rheumatoid arthritis and was treated with cortisone both preoperatively and postoperatively.

or was due to hyperparathyroidism. When PTE was injected the TRP did not decrease significantly (Fig. 4). A parathyroid adenoma was removed subsequently at operation. Ninety-one days postoperatively the initial TRP was 81 per cent and the GFR 68 ml. per minute. At this time administration of PTE decreased the TRP 8 per cent for a twenty-minute period.

DISCUSSION

Our results indicate that parathyroid extract inhibits reabsorption of phosphorus. The inhibition begins immediately and reaches its peak within sixty minutes.

In 1 subject (HA, Table 4), phosphorus excretion exceeded the filtered load by 0.20 mg. per minute after the injection of PTE. This suggests that under certain conditions PTE may increase tubular phosphorus secretion. Though other evidence for this is lacking in the mammal, tubular secretion of phosphorus is known to occur in the chicken (11).

In the past the response to PTE has usually been measured by the increase in phosphorus excretion during the three hours after injection (12). Conflicting results have been reported by investigators using this method. Much of the confusion can be attributed to the normal diurnal variation in urinary phosphorus. Phosphorus excretion is high on waking, falls to a minimum between 8 and 11 A.M. and increases to a maximum at 1 to 3 P.M. (6, 13). The spontaneous variation is so great that it may completely obscure the effect of injected PTE (13). Moreover, the degree of increase in phosphorus excretion after PTE is variable and appears to be inversely related to the initial level of excretion. These sources of error may be avoided by measuring the effect of the extract on phosphorus reabsorption between the hours of 8 and 10 A.M. As shown in our studies with Mercuhydrin, phosphorus reabsorption remains constant during this time of day.

In contrast to our observations, several investigators have reported that patients with kidney disease exhibit a diminished phosphaturic response to PTE (14, 15). Most of the latter data have been obtained by measuring the phosphorus excretion before and after injection of PTE, and are obscured by the spontaneous variation in phosphorus excretion and by the variable effect on filtration rate. When the data of Goldman and Bassett (14) are recalculated as the change in TRP, the effect of PTE is found to be similar to that observed by us. Eight of their patients with filtration rates of 141 to 53 ml. per minute responded with an average decrease of 8 per cent, compared to a decrease of 19 per cent in 7 of their patients with filtration rates of 38 to 14 ml. per minute.

The diagnosis of hyperparathyroidism in a patient with a GFR less than 49 ml. per minute poses a difficult problem. The level of TRP in the fasting

state (10) is of limited value in this situation, since it is depressed by both parathyroid and renal factors. The response to PTE in our patient was of value in differentiation. Patients with renal impairment responded with a decrease in TRP, whereas the patient with hyperparathyroidism did not respond (Fig. 4). The resistance appeared to be associated with overactivity of the patient's parathyroid gland, since a partial response occurred after removal of the adenoma.

Administration of PTE induces a generalized vasodilatation, as shown by the decrease in systemic blood pressure and increase in RPF. That the extract produced these effects in patients with pseudohypoparathyroidism and hyperparathyroidism without inhibiting phosphorus reabsorption suggests that 2 separate factors are involved. Further purification of the extract should determine whether the vasodilating factor resides in the impurities.

It is noteworthy that 2 patients with normal filtration rates and TRP of 77 per cent were being treated with triiodothyronine and cortisone, respectively. These drugs may have accounted for the low TRP value, as it has recently been shown that cortisone and triiodothyronine can depress reabsorption of phosphorus (16, 17).

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EFFECT OF PROLONGED SLEEP THERAPY AND OF CHLORPROMAZINE ON PLASMA PROTEIN-BOUND IODINE CONCENTRATION AND PLASMA THYROXINE TURNOVER*

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ABSTRACT

A study has been made of the effect of drug-induced sleep treatment on plasma protein-bound iodine (PBI) concentration and radioactive *l*-thyroxine turnover time. Ten male psychotic patients, unresponsive to conventional treatment, were put to sleep for at least twenty hours a day for thirty days with a drug combination which included chlorpromazine (600 to 2000 mg. per day), phenobarbital (240 to 360 mg. per day) sodium pentobarbital (300 to 1160 mg. per day), and Phenergan (100 mg. per day if needed). Thyroxine turnover measured during the last ten days of the sleep period was 14.8 per cent per day $\pm$ 0.4 (S.E.) as compared with 10.2 per cent $\pm$ 0.8 (S.E.) during the control period—a highly significant difference. No significant changes were observed in the plasma PBI levels. A further group of 5 psychotic patients were treated with 600 mg. of chlorpromazine per day, and were allowed to remain ambulatory. No change in thyroxine turnover occurred, and there was a small but statistically significant depression of plasma PBI concentration (from 6.8 to 5.2 μg. per 100 ml.). The finding in the sleep-treated patients suggests that plasma thyroxine turnover is not related to physical exertion, but the mechanism by which drug-induced sleep leads to a more rapid thyroxine turnover has not been elucidated. Prolonged depression of the central nervous system does not lead to depressed pituitary-thyroid function, and chlorpromazine in dosages up to 600 mg. per day also has little effect on the thyroid gland.

THYROID activity is influenced directly or indirectly by a number of homeostatic mechanisms. These mechanisms govern the rates of excretion and degradation of circulating thyroid and thyrotropic hormones, influence the character of the hormone-blood protein complexes and modify pituitary thyrotropic activity in response to changes in the external environment and the concentration of thyroid hormone in the blood. The opportunity to study in man the effect of profound nervous system depression

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and of extreme physical inactivity on certain of these functions was presented by the recently completed trial in this hospital of prolonged sleep therapy for psychosis. This treatment, widely used in European clinics and more recently studied on this continent (1), utilizes large doses of sedative and tranquilizing drugs to keep psychiatric patients asleep for long periods of time—in this study, for at least twenty hours a day over a thirty-day period.

By this study it was hoped that 2 questions might be answered. These concern the relationship of muscular activity to the rate of thyroxine degradation and the influence of the nervous system on the regulation of the pituitary-thyroid axis. If thyroxine degradation were related to physical activity it might be expected to be decreased in these inactive patients, and if the pituitary-thyroid axis were under the influence of the central nervous system, thyroid function might be expected to change in these profoundly drugged subjects.

Measurements were made therefore of the rate of plasma thyroxine turnover and the level of plasma protein-bound iodine (PBI) as influenced by thirty days of drug-induced sleep therapy. Contrary to expectations, plasma thyroxine turnover time was found to be markedly increased in the sleeping patients, whereas the PBI level was not altered significantly. Since sleep therapy led to changes in thyroxine turnover, a further group of patients were studied who were given large doses of chlorpromazine only in order to evaluate the part played by this drug in bringing about the changes observed in the sleep period.

METHODS

Male patients with schizophrenia (simple, chronic undifferentiated and paranoid) and sociopathic personality who had failed to respond to conventional psychiatric therapy were used in this study. They were in good physical condition, were neither malnourished nor senile, and had no stigmata of thyroid disease. Details of the sleep treatment will be presented elsewhere (2). The patients were given the following medications:

chlorpromazine—2000 mg. per day in fractional doses (1 patient was given 600 mg. per day),
phenobarbital—240 to 360 mg. per day in two doses,
sodium pentobarbital—300 to 1160 mg. per day in two or three doses as needed,
Phenergan—100 mg. per day in two doses, as needed.

They were kept in bed in a darkened room and awakened only for toilet and meals. Time in bed asleep was between twenty and twenty-two hours a day. Body temperature, body weight and blood pressure were observed. In 10 patients the plasma thyroxine turnover rate was measured during a control period and in the last seven to ten days of the 30-day sleep period. In 7 patients the control period of study began one month after the end of the sleep period, and in 3 the control period preceded the sleep period. No medications were given during the control period. Plasma protein-bound iodine (PBI) concentration was determined on specimens obtained during the control period and during the last three days of sleep. Similar studies were made in 5 additional patients

who were allowed to remain ambulatory while being treated with large doses of chlorpromazine. In each case control observations of plasma thyroxine turnover rate and PBI concentration were made. These patients were then given 100 mg. of chlorpromazine daily in two fractional doses for ten days; the dose was then increased to 600 mg. a day in three fractional doses. Measurement of thyroxine degradation was begun one day after chlorpromazine dosage had been increased and PBI concentration was measured eight days later.

Plasma thyroxine turnover was measured using the method of Sterling and Chodos (3). A dose of 25 to 50 μc. of I^{131}-labeled *l*-thyroxine[1] was injected intravenously and 6 to 10 serial specimens of blood were taken at intervals of one or two days beginning twenty-four hours after the injection. Plasma radioactivity was counted in a standard scintillation well counter, radioactivity decay was corrected by comparison with a standard, and the slope of the thyroxine decay curve was measured from a line calculated by the method of least squares. Plasma protein-bound iodine concentration was measured by the method of Barker *et al.* (4).

RESULTS

A. *Effects of sleep therapy*

1. *Plasma thyroxine turnover.* Mean plasma thyroxine turnover during the control period was 10.2 ± 0.8 per cent per day—a value almost identical with that found in normal (3, 5) and in psychotic subjects (unpublished observations). During the sleep period the mean turnover rate rose to 14.8 per cent per day—a highly significant change ($P < .001$). Increased turnover rate was observed in 9 of the 10 patients; in these, individual values for the increase varied between 26 and 79 per cent. In the tenth patient (Sm) the turnover rate during sleep was slightly less than during the control period. This patient was also exceptional in that his thyroxine turnover rate during the control period was very much greater than that of the others and he was one of the 3 patients who gained weight during sleep therapy (discussed later).

2. *Plasma protein-bound iodine level.* No consistent differences in PBI values between sleep and control periods were noted. Although the means during the sleep period were slightly lower, the difference was not significant ($P > .3$) and was within the range of error of the determination.

3. *General observations.* During sleep therapy the body temperature was generally normal. Mean oral temperature over the thirty-day period for each patient was between 36.5 and 36.9 C. (mean, 36.8 C.). Rarely, temperature fell as low as 35.0 C., and in 2 patients a mild upper respiratory infection was accompanied by fever up to 38.3 C., but no unusual temperatures were noted during the last ten days of the experimental period. Changes in body weight were variable (Table 1). Eight of the 10 patients lost weight (1.8 to 10.2 Kg.), and others gained (4.5 and 10.2 Kg.). Change in weight was not correlated closely with change in plasma thyroxine turn-

[1] Abbott Laboratories.

over rate, although it was noted that the patient with the greatest weight loss had the greatest increase in turnover rate during sleep, and the single subject whose thyroxine turnover rate fell during sleep was one of the 2 who gained weight during the sleep period.

TABLE 1. EFFECT OF SLEEP THERAPY ON PLASMA THYROXINE TURNOVER, LEVEL OF PLASMA PROTEIN-BOUND IODINE, AND BODY WEIGHT

Patient		Diagnosis	Thyroxine turnover (% per day)		Plasma PBI (μg./100 ml.		Change in body weight (%)
Name	Age (yrs.)		Control period	Sleep period	Control period	Sleep period	
Le	34	Schizophrenia, chronic, undifferentiated	7.6	13.6	6.5	5.5	−12.2
Th	31	Schizophrenia, chronic, undifferentiated	8.3	13.9	5.2	7.1	− 6.3
Gi	27	Schizophrenia, chronic, undifferentiated	8.5	14.1	5.9	6.3	−11.5
Pi	31	Schizophrenia, paranoid, postlobotomy	8.8	13.3	5.1	4.8	− 3.7
Tr	34	Schizophrenia, chronic, undifferentiated	9.4	15.0	5.0	5.2	+17.8
Sc	17	Personality trait disturbance, passive aggressive	9.9	16.5	5.2	3.3	− 2.6
Pi	41	Schizophrenia, chronic, undifferentiated	10.4	14.8	4.8	3.8	− 9.0
Gr	49	Schizophrenia, schizo-affective	10.8	17.0	6.3	4.9	− 7.7
Ha	35	Schizophrenia, catatonic	11.9	15.0	4.7	4.5	− 6.2
Sm	20	Schizophrenia, schizo-affective	16.3	15.1	4.9	3.7	+ 6.3
Mean ± S.E.			10.2±0.8	14.8±0.4	5.4±0.2	4.9±0.4	−3.5±2.9
Significances of differences			P<.001		P>0.3		

B. *Effect of chlorpromazine*

Plasma thyroxine turnover time and plasma PBI concentration were measured in 5 patients before and during chlorpromazine therapy. Control data were collected on a sixth patient, but observations during the chlorpromazine period were stopped when the patient contracted pneumonia, possibly related to aspiration due to drug-induced parkinsonism. The other patients showed only a slight change in behavior during treatment with 600 mg. of the drug daily, and did not have parkinsonism.

1. *Plasma thyroxine turnover.* One patient had an increased rate of thyroxine turnover, but the others showed either no significant change or a modest fall in the degradation rate (Table 2). The difference between the means was not significant (P>.4).

2. *Plasma protein-bound iodine level.* Although all values for PBI fell within the normal range, a tendency toward a lower concentration of PBI during chlorpromazine therapy was noted. All patients had a lower PBI level while receiving the drug than when not receiving it (Table 2). The difference was significant (P<.05).

DISCUSSION

In this study of psychotic men, the thyroxine turnover rate increased markedly while the plasma PBI level remained unchanged during drug-

TABLE 2. EFFECT OF CHLORPROMAZINE ON PLASMA THYROXINE TURNOVER AND PLASMA PROTEIN-BOUND IODINE LEVEL

Patient		Diagnosis	Thyroxine turnover (% per day)		Plasma PBI (μg./100 ml.)	
Name	Age (yrs.)		Control period	Chlorpromazine	Control period	Chlorpromazine
Sc	52	Schizophrenia, simple	9.9	9.3	6.8	6.1
Me	55	Schizophrenia, chronic undifferentiated	10.5	8.4	8.4	5.0
Cr	48	Schizophrenia, chronic undifferentiated	10.6	12.4	7.3	6.4
St	28	Mental defective with psychosis	12.2	12.0	6.2	4.1
We	34	Mental defective with psychosis	12.6	10.0	5.4	4.5
Mean ± S.E.			11.2±0.5	10.4±0.8	6.8±0.5	5.2±0.4
Significance of differences			P > .4		P < .05	

induced sleep therapy. Although the change in turnover rate was probably due to augmented peripheral degradation of thyroxine, the possibility of disturbed enterohepatic circulation must be considered. Increased fecal excretion is unlikely, because large-bowel motility is diminished during sleep; but abnormal accumulation of thyroxine in the quiet gut cannot be excluded *a priori*. In the normal human subject, fecal excretion accounts for less than 20 per cent of total thyroxine degradation (6, 7); in the thyrotoxic subject, this proportion falls to less than 7 per cent (7).

Rapid thyroxine turnover was an unexpected finding in these men who were at a basal level of physical activity, because the bulk of present evidence suggests that thyroxine utilization is reduced when the basal metabolic rate is depressed and is elevated when the metabolic rate is high. In hypometabolism due to myxedema, radioiodine-labeled *l*-thyroxine disappears from the blood at a subnormal rate (3, 5)—a disturbance which gradually improves after replacement therapy (3, 7). Abnormally slow turnover is also found in hypopituitarism, another disease associated with a low basal metabolic rate (5). In contrast, patients with an elevated metabolic rate due to thyrotoxicosis (3, 5, 8), leukemia (3), or salicylate ingestion (9) exhibit an abnormally fast turnover of labeled thyroxine. Comparable studies in experimental animals may be cited. Thyroxine degradation is more rapid in rats made hypermetabolic by dinitrophenol (10),

adrenaline (11), or by exposure to the cold (12, 13), and in sheep exposed to the cold by shearing (14).

Although the metabolic rate does appear to influence thyroxine degradation, it is not certain that this is true for man when variations in metabolic rate are produced by moderate changes in physical activity. In the present study the turnover rate was not slow in the sleeping patients, and in an unpublished study it was found to be normal in paraplegic patients confined to bed for many years. A moderate increase in physical activity, as in a 14-mile hike, was unaccompanied by any change in the slope of radiothyroxine disappearance curves determined in 2 normal subjects (15). On the other hand, extreme degrees of muscular exertion, such as forced swimming in rats (16) or electroshock-induced convulsions in man (17), are followed by an increase in the rate with which thyroxine disappears from the blood.

Other factors influencing thyroxine degradation that may be operative in sleep therapy must be considered. These include the possible effects of barbiturates and chlorpromazine on tissue utilization of thyroxine, and the effect of the treatment on plasma thyroxine-binding proteins. Thyroxine (8), dinitrophenol (10), and salicylates (9) all act to raise oxygen consumption and increase the fractional thyroxine turnover rate. These agents possess in common an action on mitochondria to reduce adenosine triphosphate (ATP) generation, *i.e.*, to "uncouple" oxidative phosphorylation (*cf.* 9). Recent work has shown that both barbiturates (18) and chlorpromazine (19) share a similar uncoupling property. One can only speculate that this action on intermediary metabolism, common to thyroxine, dinitrophenol, salicylates, barbiturates and chlorpromazine accounts for the changes in radiothyroxine turnover. Unlike the first 3 drugs, barbiturates (20) and chlorpromazine (21) tend to lower oxygen consumption in the whole animal, and as shown in this study, have no distinct lowering effect on the PBI level. Chlorpromazine administered alone in dosage up to 600 mg. per day was found to have no significant influence on thyroxine turnover.

Drug-induced sleep conceivably could raise the thyroxine turnover rate by lowering the level of plasma thyroxine-binding proteins—a reaction which has been observed previously in patients with certain chronic illnesses (22). The treatment causes loss of weight in most patients, and the heavy drug dosage might interfere with synthesis of blood proteins. This hypothesis cannot be established without further investigation, but it should be noted that there was no correlation between the degree of weight loss and the change in turnover rate in this study.

Inhibitory effects of chlorpromazine on pituitary-thyroid activity, as inferred from turnover and PBI data, were not marked or consistent. This

negative finding sheds no light on the problem of neural control of thyroid function in the human subject. Other tranquilizing drugs such as reserpine (23, 24, 25), promazine (25), meprobamate (25) and hydroxyzine (25) are similarly ineffective in reducing thyroid function in man.

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EFFECT OF HYPNOTICALLY-INDUCED ANXIETY ON THE PLASMA HYDROCORTISONE LEVEL OF NORMAL SUBJECTS

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ABSTRACT

Twelve normal subjects were made anxious by direct suggestion while in a deep, somnambulistic state produced by means of hypnosis. The trends in plasma hydrocortisone level throughout the waking, hypnotized, anxious, and waking periods were significantly different between the female subjects and their controls, but not between the males and their controls. Although hypnosis significantly reduced the plasma hydrocortisone level in both sexes, induction of anxiety raised the level in the female subjects by 75 per cent but did not affect the male subjects. A statistical treatment for trend analysis and comparison has been presented which may be helpful in similar studies.

A VARIETY of experimental (1–3) and natural (4–6) situations have been employed in order to investigate the relationship between emotional arousal and elevation of adrenocortical activity in man. The plasma level of hydrocortisone in human subjects following the application of a stress stimulus generally has not reached that found in severely anxious patients (7) nor in men whose adrenal glands were maximally stimulated by the administration of large doses of corticotropin (ACTH) (8,9). Occasionally, some subjects responded to the stimulus with an increase of up to 500 per cent in the plasma hormone level. The failure of most subjects to respond rapidly to a variety of psychologic stimuli with large increases in the level of plasma hydrocortisone may have been due to any of the following 3 factors: 1) the stimulus employed was not sufficiently meaningful in the specific case to serve as an anxiety-eliciting signal; 2) the intensity of the stimulus was not maintained at a sufficiently high and/or constant level; or 3) the investigator (a physician or psychologist) was reluctant to increase the stimulus to its maximum because of the potential or actual danger to the health of the subject.

In order to minimize these objections and induce a consistent rise in the hydrocortisone level in human subjects, we chose to employ a hypnotically-induced anxiety state which was subsequently abolished and followed by

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the induction of a profound amnesia for the experimental procedure. By this technique, we achieved greater increases in the level of plasma hydrocortisone in female subjects with no apparent deleterious effects and with a higher degree of consistency than has hitherto been obtained by other methods.

SUBJECTS, DESIGN OF STUDY, AND METHODS

The subjects of the present study were 12 paid volunteers (6 men and 6 women) who were students at the University. The average age was 22.9 years (range, 18–29 years). They were selected from a larger group of volunteers by means of a careful screening procedure designed to procure subjects who were: 1) physically amd mentally healthy, 2) relatively devoid of current anxiety and anxiety-proneness, 3) capable of achieving a deep somnambulistic state under hypnosis, and 4) capable of developing a total amnesia for the experimental procedures while under hypnosis.

The hypnotic induction of the anxiety state was achieved by an elaboration of the method of Erickson (10) as modified by Levine, Grassi and Gerson (11). A standardized set of instructions suggesting that the subject should experience feelings of anxiety were read to each of the group, with appropriate emphasis. This stimulus was remarkably similar in each case, as the same word content was employed and the tonal quality was maintained at approximately the same level. Differences in response among subjects did occur and required minor adjustment in technique, such as an occasional repetition of a sentence or two. In order to maintain a relatively high level of affect, additional reinforcement was required toward the end of the experimental anxiety phase. The intensity of anxiety experienced varied from subject to subject, but all of them experienced some degree of anxiety, as manifested by verbal communication of a feeling of imminent danger, discomfort and/or nervousness, as well as by such somatic symptoms and behavioral manifestations as crying, nasal congestion, alteration in heart rate and respiration, palmar sweating, increased restlessness and muscle tension. No difference in the level of anxiety between the sexes was observed.

Blood samples for the determination of plasma hydrocortisone and several other substances were drawn from each subject prior to hypnosis, following the induction of the hypnotic trance, following the induction of the anxiety state, and after termination of the trance. These occasions corresponded approximately in time to the samples drawn from the controls at 9:00 A.M., 10:30 A.M., 12:00 noon and 1:30 P.M. During each part of the study, a Rorschach test was administered.

The 12 subjects were unable to serve as their own controls because of their limited available time. Consequently, 12 additional subjects, matched for sex and age, were selected to serve as controls. These persons were in good physical and mental health but were not subjected to the tests for the assessment of hypnotizability. The sole function of this group was to provide data concerning the variability of hydrocortisone levels in plasma obtained at the same times as from the anxious subjects.

Plasma hydrocortisone concentration was determined by the method of Nelson and Samuels (12, 13). The degree of emotional arousal of the subjects who were hypnotized was estimated by 2 observers during the entire study.

RESULTS

The plasma hydrocortisone levels and the standard deviations on the 4 occasions of measurement in each group of the anxious subjects are listed

in Table 1. The plasma levels for the control subjects measured at comparable times on two widely separated days are listed in Table 2. A test for the existence of a trend in hormone level is included in each table. In the past, in order to determine the effects of various procedures on the level of plasma hydrocortisone—a variable known to undergo diurnal variation (14)—we corrected the post-experimental measurement by adding an average "correction factor" derived by measuring the plasma levels in a group of control subjects at times comparable to the pre- and post-experimental

TABLE 1. PLASMA HYDROCORTISONE LEVELS OF 12 SUBJECTS WITH HYPNOTICALLY-INDUCED ANXIETY, ON 4 TESTING OCCASIONS

Subjects	No.	Plasma hydrocortisone level (μg./100 ml.)*			
		Pre-hypnosis	Hypnosis	Anxiety+Hypnosis	Post-hypnosis
Males	6	22.5±4.7	13.2±7.3	12.1±5.9	10.4±7.9
Females	6	19.3±8.1	12.5±3.8	20.2±4.7	12.9±6.0
Both	12	20.9±6.5	12.9±5.5	16.2±6.6	11.7±6.8

Tests for Existence of Trend

Source of variation	df	Males		Females	
		Mean sq.	F	Mean sq.	F
Between times of day	3	175.42		99.78	
Error†	15	38.34	4.57‡	18.03	5.53§

* Mean and standard deviation.
† Time of day×subject interaction.
‡ Significant at better than the 5% level.
§ Significant at better than the 1% level.

occasions (5, 15). However, considerable misgiving developed regarding this procedure and we have re-examined the problem of determining the effect of one or more treatments on a non-stable variable. In our previous "correction" formula we assumed that the individual trend in level during the day was relatively constant and we did not take into account the variability of the control group's diurnal pattern. In order to validate these 2 assumptions, we measured the plasma hydrocortisone levels in the control group at times comparable to the four occasions in the anxiety group on two widely separated days (Table 2). Calculation of the variance due to variation in trend from day-to-day in the same subject (variance due to the interaction of day×time of day within subject) and variance due to variation in average trend from subject to subject (time of day×subject) revealed a number of important facts (Table 3): 1) individual trend in level was more variable than we had anticipated; 2) there was no greater stability of trend from one day to another in the same subject than there was from one subject to another; and 3) in both variance estimates, the females exhibited about double the instability in trend manifested by the males

TABLE 2. PLASMA HYDROCORTISONE LEVELS OF 12 CONTROL SUBJECTS ON 4 TESTING OCCASIONS ON TWO SEPARATE DAYS

Subjects	No.	Plasma hydrocortisone level (μg./100 ml.)*											
		First day				Second day				Average			
		9:00 A.M.	10:30 A.M.	12:00 NOON	1:30 P.M.	9:00 A.M.	10:30 A.M.	12:00 NOON	1:30 P.M.	9:00 A.M.	10:30 A.M.	12:00 NOON	1:30 P.M.
Males	6	16.2±4.5	11.2±2.8	11.7±4.0	11.3±4.8	17.7±5.6	15.0±5.8	14.5±3.9	12 6±8 5	16.9±2.4	13.1±2.6	13.1±3.0	11.9±4.3
Females	6	13.9±8.0	14.1±3.9	12.0±2.5	10.6±2.8	13.3±5.8	10.8±5.4	9.5±5.0	10.4±8.5	13.6±4.4	12.5±3.1	10.7±2.5	10.5±4.5
Both	12	15.0±6.3	12.6±3.6	11.8±3.2	10.9±3.8	15.5±5.9	12.9±5.8	12.0±5.0	11.5±8.2	15.2±3.8	12.8±2.7	11.9±2.9	11.2±4.3

Tests for Existence of Trend

Source of variation	df	Males		Females	
		Mean sq.	F	Mean sq.	F
Between times of day	3	57.11		25.50	
Error†	33	12.97	4.40‡	25.97	0.98

* Mean and standard deviation.
† From last column of Table 3.
‡ Significant at better than the 5% level.

(in contrast to the subjects of the anxiety study in whom the exact opposite was true). When the sources of variation were pooled, the difference between the sexes achieved the 5 per cent level of significance. The meaning of this difference is not readily apparent. These findings suggest that it is unwise to use average "corrections" without taking into accout their error variances.

Two alternative methods for comparing the effects of the various procedures on the level of plasma hydrocortisone were consequently devised.

TABLE 3. VARIABILITY OF DIURNAL TRENDS IN PLASMA HYDROCORTISONE LEVELS OF 12 CONTROL SUBJECTS

Subjects	Source of variation					
	Subject-to-subject		Day-to-day within subjects*		All trends	
	df	Mean sq.	df	Mean sq.	df	Mean sq.
Males	15	11.57	18	14.13	33	12.97
Females	15	20.76	18	30.31	33	25.97
Both	30	16.16	36	22.22	66	19.46
Comparison of males with females, F	1.79		2.14		2.00†	

* Contributions from each subject to these mean squares were tested for heterogeneity using the Bartlett test. Nonsignificant chi-squares were obtained in all cases.

† Significant at better than the 5% level. A two-tailed F-test rather than a single-tailed test is used when comparing variances from 2 independent groups.

The methods are related, as will be seen subsequently, and are applicable to any variable which undergoes diurnal fluctuation. The first and more general procedure compared the average trend in hormone level for the 4 testing occasions in the anxiety group with the average trend in the control group. In order to compare average trends, it is desirable first to determine whether a true average trend exists. In Tables 1 and 2, the F-tests indicated that a true average trend existed for the male and female subjects of the anxiety group and for the male subjects of the control group. Inspection of the means in Table 2 for the female control subjects, together with previous experience with this variable, led us to believe that a true downward average trend probably also existed for the female subjects, but the variability of the female trends was so great that we were unable to demonstrate that it was statistically significant using the data at hand.

The departure of the average trend in the anxiety group from that in the control group was assessed for statistical significance by a test of the inter-

TABLE 4. COMPARISON OF AVERAGE TRENDS IN PLASMA HYDROCORTISONE LEVELS FOR ANXIETY AND CONTROL GROUPS*

Comparison	Approx. df for denominator	F′‡
Anxious males *vs.* control males	20	1.41
Anxious females *vs.* control females	36	3.25§
Anxious males *vs.* anxious females	27	2.55
Control males *vs.* control females	59	0.43

* These comparisons are made by a test which does not assume homogeneity of error variance. The control males and females each have 12 trends (n) and 33 degrees of freedom for their error variance; the anxious males and females each have 6 trends and 15 degrees of freedom for their error variance. The trend variability (s^2) for each group is the appropriate error term of Tables 1 and 2.

‡ This F′-test is carried out as follows: for the 2 groups whose average trends are to be compared, the difference is computed between the means of each group at each time; the variance of these differences is then computed, and employed as the numerator of F′, with 3 degrees of freedom in each case. The denominator of F′ is obtained from the trend variability variances as follows:

$$\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}$$

The approximate degrees of freedom for the denominator are computed by a rule apparently first suggested by Satterthwaite (16). The rationale for such F′-tests has been given by Cochran (17).

§ Significant at better than the 5% level.

action, group×time of day. The comparison was performed separately for each sex because of the significant differences in their error variances. The results of the comparisons are listed in Table 4. The average trend for the females of the anxiety group was significantly different from that for their controls, but no similar differences occurred for the male subjects. This finding is discussed more fully after the presentation of Table 5. No significant difference in average trends between the sexes occurred within the anxiety or the control groups.

The second method for comparing the effects of the various experimental procedures on the level of plasma hydrocortisone is related to the preceding comparison of trends, but assesses more specifically the significance of the differences in hormone levels for the various adjacent time intervals. This method employs the average correction mentioned earlier but takes into account the error variance of the correction. By means of this procedure, a more detailed picture of the effect of the hypnotically-induced anxiety may be drawn than is obtained from the comparison of average trends. In the upper part of Table 5, the mean changes in plasma hydrocortisone level for adjacent occasions are listed for each group of subjects.

These intervals represent changes in the anxiety group from the waking state to the hypnotized basal state (9:00 to 10:30), from the hypnotized basal state to the hypnotized anxious state (10:30 to 12:00), and from the hypnotized anxious state to the waking state respectively (12:00 to 1:30). Any change value which exceeds the calculated value in the last column of Table 5 is significant at better than the 5 per cent level. For both males and females, induction of the hypnotic state significantly reduced the plasma hydrocortisone levels from the initial waking values. This significant reduc-

TABLE 5. CHANGES IN THE MEAN PLASMA HYDROCORTISONE LEVEL BETWEEN ADJACENT OCCASIONS

Group	Mean change in plasma hydrocortisone level from:			Estimated standard error of mean change, $\sqrt{2s^2/n}$	Least significant change at 5% level*
	9:00 to 10:30	10:30 to 12:00	12:00 to 1:30		
Control males	−3.8	0.0	−1.2	1.47	±3.0
Control females	−1.1	−1.7	−0.2	2.08	±4.2
Anxious males	−9.3	−1.1	−1.7	3.57	±7.6
Anxious females	−6.7	7.7	−7.3	2.45	±5.2

Comparisons of Changes between Adjacent Occasions in the Mean Plasma Hydrocortisone Level

Groups compared	Difference in change from:			Estimated standard error of difference in mean change, $\sqrt{2(s_1^2/n_1+s_2^2/n_2)}$	Approx. df	Least significant difference at 5% level†
	9:00 to 10:30	10.30 to 12:00	12:30 to 1:30			
Control males *vs.* anxious males	5.5	1.1	0.5	3.87	20	±8.1
Control females *vs.* anxious females	5.6	−9.4	7.1	3.22	36	±6.5
Anxious males *vs.* anxious females	−2.5	−8.8	5.6	4.34	27	±8.9
Control males *vs.* control females	−2.7	1.7	−0.9	2.55	79	±5.1

* Obtained by multiplying the standard error by the two-tailed 5% level of "t" with the appropriate degrees of freedom. In the top part of the table, these are 15 for anxious males and females, and 33 for controls. In the bottom part the appropriate degrees of freedom are given in the next-to-last column. This method does not permit one-tailed tests in these cases when such tests would have been appropriate because of *a priori* prediction. However, the conclusions reached would not be affected by application of one-tailed tests in these instances.

† Computed by the rule suggested by Satterthwaite (16).

tion had been anticipated, since the induction of the hypnotic state was accompanied by a suggestion of increased relaxation and tranquility. The reduction in plasma hormone level following the onset of hypnosis was at least twice as great as that observed over the comparable time-span in the nonhypnotized controls (41 *vs.* 22 per cent for males, and 35 *vs.* 8 per cent for females). The induction of an anxiety state significantly raised the hormone level in the female subjects by 75 per cent above the 10:30 A.M. value. It did not, however, produce a similar effect in the male subjects (with 1 exception). Following the termination of the hypnotic trance, the plasma hydrocortisone level in the female subjects rapidly and significantly decreased almost to the level in the male subjects. The difference in the plasma hydrocortisone response to the anxiety stimulus between the male and female subjects was a result of factors which will be treated more fully in another publication.

The female subjects not only reacted to the anxiety stimulus with the anticipated response, but responded to the entire procedure in an ordered fashion. Each one tended to maintain her position relative to the other members of the female group with respect to hormone level. This was not observed in the male subjects (coefficient of concordance, $W = 0.71$, $P < 0.01$). The similarity in the responses of these female subjects was noted previously in their lower trend variability estimate (error term in Table 1). By contrast, among the control subjects, it was the males who had the greater tendency to maintain their level relative to the group across all occasions, as shown in their trend variability estimates (Table 3) and their coefficient of concordance ($W = 0.30$, $P < 0.05$).

A comparison of the change in hormone level for adjacent occasions between anxiety and control subjects is shown in the first two lines of the bottom half of Table 5. The least significant differences in the last column are used in a manner similar to that for the upper half of Table 5. These comparisons are actually tests of significance of differences of portions of the average trends compared in Table 4. In that table, male subjects of the anxiety group were shown not to differ significantly from their controls; likewise, these 2 groups did not differ significantly in any of the 3 individual mean changes. By contrast (Table 4), the female subjects of the anxiety group differed significantly from their controls with respect to their average trends, and they also differed significantly with respect to the mean change from 10:30 A.M. to 12:00 noon, and from 12:00 noon to 1:30 P.M. That is, anxiety-induction and hypnosis-termination both produced significantly greater mean hydrocortisone changes than diurnal variation produced in the control subjects. In the third line of this table, a comparison of mean changes in female and male anxious subjects indicates a significantly greater increase for the former group under the anxiety stimulus.

DISCUSSION

The experimental production of emotional states accompanied by physiologic changes usually associated with such states in real life provides a useful method for the investigation and validation of postulated mechanisms presumed to be important in the area of psychosomatic interrelationships. Of the many methods employed for this purpose in the human subject, hypnosis probably provides one of the most effective techniques, involving as far as can be determined, the least amount of psychologic trauma. Several workers have summarized the evidence and arguments favoring the use of hypnosis as a tool for the investigation of psychosomatic processes (17–21). Gorton (18) points out that affective arousal can be rapidly induced under hypnosis and that the character of the emotion produced differs in no important consideration from affect generated in other investigative situations. The observations on our subjects adequately confirm this statement; their verbal communication of fears of unknown origin and their somatic and behavioral manifestations of distress usually exceeded the expression of similar emotion by subjects in a real, life-threatening situation such as paratroop training (22) or by anxious patients whose nuclear conflicts were stirred up by means of a disturbing interview (5).

The induction of an anxiety state during hypnosis not only produced manifest anxiety of considerable intensity, but also produced an important physiologic concomitant—an elevation of the plasma level of hydrocortisone. The mean increase in hormone level amounted to 75 per cent for the female subjects, an amount greater than that reported for any other psychologic technique cited in this paper. The failure of the male subjects to respond to the suggestion of anxiety with a significant increase in hormone level was probably due to characteristics of the experimental design and subject selection rather than to a lack of effect of the stimulus *per se;* in other anxiety-inducing studies, *e.g.*, a disturbing interview (23), there was no such difference between sexes. That hypnosis is an effective evocator of anxiety and its concomitants is not a unique finding; long ago Wittkower demonstrated profound adrenocortical activation in man under the stimulus of hypnotically-induced anxiety using the then-available index of the leukocyte count (24).

The present study points up the speed with which the adrenal cortex responds to a disturbing stimulus, a fact frequently noted in other reports. The rise in the level of plasma hydrocortisone occurred within ninety minutes of the anxiety suggestion. Likewise, termination of the anxiety state led to an equally rapid decline in the hormone level.

In contrast to the effect of hypnotically-induced anxiety on the plasma hydrocortisone level, hypnosis alone led to a significant reduction in hormone level in both male and female subjects. It is not possible to establish with the present data whether this reduction was a direct effect of hypnosis

per se or a consequence of the hypnotic induction techniques used.

The reduction in anxiety and hydrocortisone level following the induction of the hypnotic state points up the initially elevated plasma hormone levels in these subjects. The levels obtained at 9:00 A.M. were equivalent to those seen in hospitalized, anxious patients. Although the subjects were selected for their relatively low anxiety level and low anxiety-proneness, on the day of testing their plasma hormone levels were high. This elevated level may have reflected certain features of the experimental design which were unavoidable. The subjects were told prior to the day of testing that the study involved the relationship of emotions to blood chemistry, but no further details were given. Although they were trained to become good hypnotizable subjects, they did not know what the procedures were to be. It is not unlikely, therefore, that a good deal of current anxiety may have developed immediately before the start of the experiment. The anticipation of potential danger in a strange setting has been shown to evoke elevated plasma hydrocortisone levels in man (5), and similar results have been demonstrated even in a familiar setting in monkeys (25).

The present study has also provided a useful statistical procedure for the comparison of *trends* in the level of a variable. Such mathematical comparisons are not common in biochemical or physiologic investigations in which at best, differences in *level* or in *change of level* are assessed. When trends are compared, graphic presentation and visual inspection are employed—techniques which are less reliable than statistical tests. In the present instance, the use of a trend-in-level variability estimate suggested a number of future data analyses intended to measure degrees of physiologic stability not readily ascertainable by gross inspection of the data.

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REPOSITORY FORMS OF ADRENOCORTICOTROPIN (ACTH)

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ABSTRACT

The duration of activity of long-acting repository ACTH preparations depends somewhat upon the hormone requirement of the patient and appears to vary with the method employed for its estimation. Following injection of 40 U.S.P. units of corticotropin-zinc, the increased level of plasma 17-hydroxycorticosteroids returns to normal within about twenty-four hours, the urinary sodium/potassium ratio and the blood eosinophil count remain depressed, and urinary corticosteroid excretion remains elevated for twenty-four to seventy-two hours, while the clinical response may last as long as a week. By each criterion, ACTH combined with alpha-zinc hydroxide is more potent and has a longer duration of action than the same amount of ACTH in gelatin. Doubling the dose tends to increase slightly the duration of action.

THE clinical usefulness of the adrenocorticotropic hormone in about one hundred diseases has stimulated the search for repository ACTH preparations. Of the numerous preparations of this type which have been proposed, only 2 have achieved practical importance in the United States. These are the solution in gelatin (1) which was introduced in 1952, and the combination with alpha-zinc hydroxide (2) which became available in 1955. Incorporation of ACTH into a 16 per-cent solution of gelatin increases the duration of its action after intramuscular injection to eighteen hours, as compared with only six or eight hours for ACTH in saline. In addition, the total clinical effect of the same amount of ACTH is increased about threefold by incorporation into the gelatin repository medium. A preparation with still longer action was introduced in Europe in 1953; this two-vial substance, on admixture, produced a suspension of corticotropin adsorbed on colloidal zinc phosphate. Further research made possible the introduction in the United States in 1955 of a fluid, stable, one-vial suspension containing 40 U.S.P. units of highly purified corticotropin and alpha-zinc hydroxide corresponding to 2.0 mg. of zinc per ml.

The potency of ACTH preparations is measured by their ability to deplete adrenal ascorbic acid (Sayers' test) or to stimulate *in vitro* the produc-

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tion by the adrenal of corticosteroids (Saffran assay), but other methods are used to evaluate the long-acting repository form. Thus, depression of the eosinophil count following a single injection of ACTH lasts from twelve to twenty-four hours when the hormone is in gelatin, and twenty-four to forty-eight hours when the same amount of hormone is combined with alpha-zinc hydroxide (3). Urinary excretion of 17-hydroxycorticoids remains elevated for thirty-two hours following injection of 40 U.S.P. units of zinc-corticotropin in healthy males, whereas essentially normal values are obtained sixteen hours after injection of 40 units of corticotropin-gel (4–6).

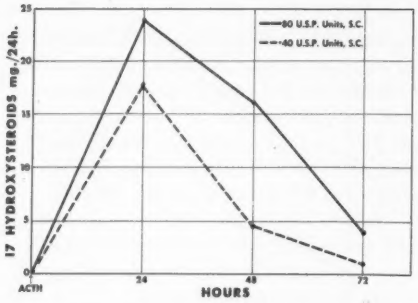

FIG. 1. Increment over normal of urinary 17-hydroxycorticosteroids excreted up to 24, 48 and 72 hours after injection of 40 and 80 U.S.P. units of corticotropin alpha-zinc hydroxide in patients with lupus erythematosus. (Personal communication, P. H. Forsham.)

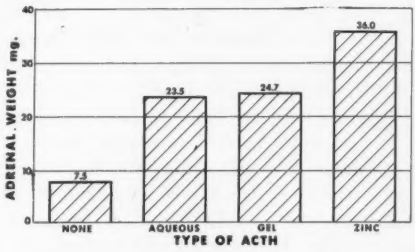

FIG. 2. Adrenal weights in 30-day-old hypophysectomized Sprague-Dawley rats given 4 units of ACTH daily as the aqueous solution, the gel form, or with alpha-zinc hydroxide for nine days following operation. The average total adrenal weight in 60 control rats (hypophysectomized but given no ACTH) was 7.5 mg.; in those given aqueous ACTH (6 animals), 24.7 mg.; in those given gel ACTH (4 animals), 23.5 mg.; in those given alpha-zinc hydroxide ACTH (6 animals), 36.0 mg. (Personal communication, J. Jailer).

Figure 1 (Forsham) shows the increased excretion of urinary 17-hydroxycorticosteroids for forty-eight to seventy-two hours after injection of ACTH alpha-zinc hydroxide in patients with lupus erythematosus; similar studies with gelatin-ACTH show increased excretion for eighteen to twenty-four hours. Plasma corticosteroid levels likewise remain elevated for a significantly longer period following injection of zinc-ACTH than after the same amount of ACTH in gelatin. High levels of plasma 17,21-dihydroxy-20-ketosteroids are found two hours following injection of either the gel or zinc forms, but after eight hours the high levels are found only in those patients receiving zinc-ACTH (Geller, Gabrilove and Soffer (7)).

Probably because of the slow release into the bood stream of ACTH

from its insoluble complex with alpha-zinc hydroxide and because of the protective action of zinc against destruction of ACTH by proteolytic enzymes, the same amount of ACTH stimulates production of almost twice the amount of corticosteroids and has almost twice the clinical effect when combined with alpha-zinc hydroxide than when dissolved in gelatin. Figure 2 (Jailer) illustrates the greater effectiveness of alpha-zinc hydroxide ACTH in maintaining adrenal weights in rats after hypophysectomy. In Figure 3 (Forsham) is shown the greater urinary excretion of 17-hydroxycorticosteroids during the 48-hour period after injection of 20, 40, 60 and

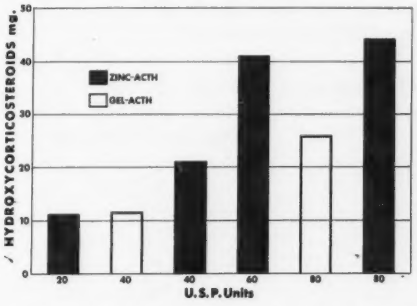

FIG. 3. Total urinary 17-hydroxycorticosteroid excretion *above normal* period during a forty-eight hours following injection of 20, 40, 60 and 80 U.S.P. units of ACTH alpha-zinc hydroxide and 40 to 80 units of ACTH gelatin. Data on 40 units are the average for 6 normal males given injections of gel- and zinc-ACTH. Patients given 20 to 60 units were normal adult males. Data on 80 units are for patients with lupus erythematosus. (Personal communication, P. H. Forsham.)

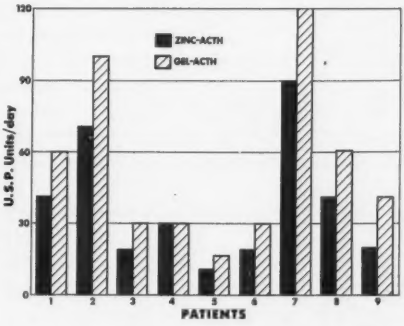

FIG. 4. Minimum amounts of ACTH gelatin and ACTH alpha-zinc hydroxide necessary to maintain equivalent response in patients with rheumatoid arthritis and disseminated lupus erythematosus. (Adapted from data of Banghart, H. E. and Watanabe, R. K. D. (8*b*)).

80 U.S.P. units of ACTH combined with alpha-zinc hydroxide, as compared with 40 and 80 U.S.P. units of ACTH in gelatin. Figure 4 (Banghart) shows a comparison of the minimum amounts of ACTH in the gelatin and in the alpha-zinc hydroxide forms necessary to maintain a sustained remission in patients with rheumatoid arthritis and collagen vascular diseases. In these patients, 43 units of zinc-ACTH was more than equivalent to 61 units of gel-ACTH (8). Other clinicians have found zinc-ACTH to be proportionately more effective (3).

Recent research on the nature of the corticotropin-zinc hydroxide complex has shown that of the 6 or more crystalline forms of zinc hydroxide only the alpha form, which has a lattice structure, combines with adreno-

corticotropin and that this form is produced in 100 per cent yield by electrolysis of a zinc salt in the presence of ACTH. By this new electrolytic process (Lewin (9)) a stable, well tolerated corticotropin alpha-zinc hydroxide supension of very small, uniform particles can be prepared with only 1.0 mg. of zinc per 40 U.S.P. units of ACTH. Comparison of blood 17-hydroxycorticosteroid levels eight hours after injection of this electrolytic preparation (Table 1) with those obtained previously for the commercial preparation made by chemical precipitation (7) with 2.0 mg. of zinc per ml.

TABLE 1. PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS INITIALLY AND EIGHT HOURS AFTER INJECTION OF 40 U.S.P. UNITS OF CORTICOTROPIN ALPHA-ZINC HYDROXIDE CONTAINING 1.0 MG. OF ZINC

Patient	Plasma hydrocortisone level (μg./100 ml.)*	
	Initial	8 hrs. after injection
A	19.7	54.7
B	9.0	47.2
C	17.1	43.4
D	18.1	47.8
E†	20.9	79.3
F‡	15.7	48.7
G	25.1	51.6
H	24.0	63.0

* Silber-Porter method.

† The plasma level 24 hours after injection was 24.8 μg./100 ml.

‡ The plasma level 24 hours after injection was 25.0 μg./100 ml.

shows at least equivalent, and perhaps somewhat greater potency. Extensive clinical evaluations established that the relatively painless injections of the electrolytic preparation produced at least equivalent and perhaps somewhat greater clinical results than the previous material. The effects were prompt (because of the 5 per cent uncombined ACTH) compared with those produced by six-hour intravenous infusions. A patient with rheumatoid arthritis who had become refractory to injections of other ACTH preparations again responded to electrolytic zinc-ACTH, perhaps because of the higher purity of the ACTH.

Urinary excretion of 17-ketosteroids, sodium and potassium, and blood eosinophil counts have been determined following single injections of 40 U.S.P. units, and twenty days later following 80 units of electrolytic corticotropin-zinc containing 1.0 mg. of zinc per ml. (McGavack (10)). The patients were 12 elderly women (aged 63 to 84) with diabetes mellitus, on a

metabolic ward. No changes in the control of diabetes or the clinical status were noted following injection of corticotropin-zinc. Urinary excretion of 17-ketosteroids was increased in all subjects for forty-eight to seventy-two hours after 40 U.S.P. units and in 8 of 10 subjects for forty-eight to ninety-six hours after 80 units (Table 2, McGavack).

The ratio of sodium to potassium in the first specimen of urine each morning was decreased twenty hours after administration of 40 units of corticotropin-zinc in 9 of McGavack's 10 subjects, and for twenty to forty-four hours after 80 units in 8 of the 10. Eosinophil counts were decreased

TABLE 2. URINARY 24-HOUR EXCRETION OF 17-KETOSTEROIDS BEFORE AND AFTER INJECTION OF 40 UNITS OF CORTICOTROPIN-ZINC*

Subject	Urinary 17-ketosteroids (mg./24 hrs.)				
	Control values		Hours after injection of ACTH-zinc		
			24	48	72
1	†	2.3	15.1	20.8	13.7
2	2.3‡	2.8‡	8.3	3.4	4.9
4	1.0	1.1	4.1	3.1	2.2
5	2.9	†	3.1	8.8	7.0
6	4.8	4.0	9.2	5.3	7.4
8	3.3	†	8.2	9.0	5.4
9	2.6	2.0	5.1	†	3.9
10	†	3.8	9.7	12.1	6.4
11	†	2.5	10.6	5.1	2.8
12	1.3	†	2.2	†	1.0

* Data of T. H. McGavack, M.D.(10).

† Specimen not obtained or incomplete.

‡ Original mean control value, 1.5 mg./24 hrs.

for twenty-four to forty-eight hours after injection of 40 units of corticotropin-zinc in 6 of the 10 subjects, and for twenty-four to seventy-six hours after 80 units in 5. In some patients, nineteen or twenty days after 40 units of corticotropin-zinc, urinary 17-ketosteroids remained higher and the sodium/potassium ratio and eosinophil count remained lower than the original control values, possibly indicating increased adrenal function stimulated by successive doses of ACTH.

Acknowledgment

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THYROID ADENOMAS AND THYROTOXICOSIS IN PATIENTS WITH HYPOPITUITARISM FOLLOWING HYPOPHYSECTOMY

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ABSTRACT

Development of thyroid adenomas has been observed in 2 hypophysectomized women, one of whom became severely thyrotoxic. In neither patient was there evidence of function of the anterior pituitary or of any target-organ other than the thyroid. Under certain circumstances thyroid adenomas appear to be autonomous in their growth and function and are able to synthesize normal or excessive amounts of thyroid hormone in the absence of thyroid-stimulating hormone. Thyrotoxicosis is not necessarily a pituitary-induced disorder.

MAINTENANCE of thyroid gland function is usually considered to be dependent upon the secretion of thyroid-stimulating hormone (TSH; thyrotropin) by the anterior lobe of the pituitary. Pituitary insufficiency, whether total or due to isolated failure of TSH secretion, is as a rule accompanied by secondary hypothyroidism,myxedema being an important sign of pituitary failure. Conversely, the injection of TSH and probably also endogenous hypersecretion will induce hyperthyroidism. These concepts of thyroid physiology should be re-examined in order to reconcile the paradox of pituitary hypofunction occurring in association with thyroid hyperfunction. We have come across 2 patients with pituitary insufficiency following surgical hypophysectomy in whom thyroid adenomas developed or increased in size; one of these patients became thyrotoxic.

Similar instances in which this unusual combination has occurred have recently been reported (1–3).

METHODS

Surgical hypophysectomy was carried out through a right frontal approach, the pituitary stalk being divided by diathermy and the gland parenchyma removed piecemeal as completely as possible before placing 5 unscreened Au^{198} seeds embedded in fibrin plaques in the empty fossa (4). Preoperative studies were carried out after giving 40 μc. of I^{131} by mouth, or 60–75 μc. postoperatively. The level of serum protein-bound iodine was measured at forty-eight hours after precipitation with trichloracetic acid. Standard procedures were used for clinical chemical analyses. Modifications of the

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following methods were used for other investigations: urinary 17-oxosteroids, Drekter *et al.* (5); urinary 17-hydroxycorticosteroids, Reddy, Jenkins and Thorn (6); plasma 17-hydroxycorticosteroids, Nelson and Samuels (7) and Gurling, Gore and Baron (8); urinary mammotropic activity, Hadfield and Young (9); urinary gonadotropin, a mouse-uterus assay after kaolin-acetone extraction (I. F. Sommerville); urinary estrogens, Brown, Bulbrook and Greenwood (10).

CASE I

Before hypophysectomy—July 1956

Clinical history. Mrs. G.C., aged 52 years, noted a lump in the left breast in June 1954. In January 1955 she received radiotherapy for an inoperable spheroidal-cell carcinoma. In April 1956 bilateral pleural effusions developed, the breast tumor increased in size, and she was advised to undergo hypophysectomy in July 1956. Her general condition was fair, and her weight was 117 pounds (53 Kg.). She was very dyspneic, however, due to the pleural effusions, and there was a large fixed mass in the left breast with lymph-node metastases in the axilla and supraclavicular fossa. She had a vivacious appearance, but there were no clinical signs of thyroid hyperfunction. The resting pulse was between 74 and 84 per minute, the thyroid was normal in size and consistency, there were no abnormal eye signs and she had no tremor. On July 24, 1956 she underwent hypophysectomy. Removal of the gland appeared to be complete and postoperative recovery was rapid.

Laboratory investigations. Thyroid function appeared to be normal. The results of relevant investigations are summarized in Table 1. Adrenal function was also normal as judged by urinary and plasma steroid values (Table 2). Urinary gonadotropin was present at the 96 m.u. level, but estrogens were not determined. Other findings were: hemoglobin level 13.6 Gm. per 100 ml.; serum alkaline phosphatase 9.5 King-Armstrong units per 100 ml.; and serum calcium 10.8 mg. per 100 ml. Results of glucose and glucagon tolerance and insulin sensitivity tests were normal.

After hypophysectomy—July 1956 to June 1957

Clinical history. After hypophysectomy her condition improved rapidly and within three weeks the mass in the left breast and the lymph nodes had become smaller and the pleural effusions had decreased. Diabetes insipidus was troublesome, though controlled by Pitressin snuff. With a daily maintenance dose of 37.5 mg. of cortisone she felt very well. Biopsy of a cutaneous nodule three weeks after operation showed that in comparison with the histologic appearance before operation the tumor cells were fewer in number and their nuclei had become pyknotic. The remarkable and rapid clinical improvement provided strong evidence that hypophysectomy had been complete. There were no clinical manifestations of hypothyroidism by the fourth week, and because the results of thyroid function tests did not indicate a significant reduction in activity, no oral thyroid was given. All the investigations were repeated in the tenth week. Definite clinical and laboratory evidence of imparied adrenal function was obtained during the 72-hour cortisone withdrawal test. The patient felt tired and weak and lacked appetite. These typical symptoms of glucocorticoid deficiency were relieved immediately by cortisone therapy.

Laboratory investigations. The changes in thyroid function are shown in Table 1. Although four weeks after operation there was a reduction in the level of PBI^{131} as compared with the preoperative value, the 24 per cent thyroidal uptake and 49 per cent urinary excretion at twenty-four hours were unusually high for a completely hypophy-

sectomized patient. By the tenth week the thyroidal I^{131} uptake had fallen, the urinary excretion had increased and no PBI^{131} could be detected in the plasma. Since the BMR was now −22 per cent (Aub-Du Bois) and the serum cholesterol level 280 mg. per 100 ml., it was accepted that thyroid function had been impaired. Thyroid medication was started. Urinary gonadotropin excretion had fallen to less than 24 m.u. per twenty-four hours, the extract being toxic at the 6-unit level. During the 72-hour cortisone withdrawal test, urinary 17-oxosteroid excretion declined to 1.4 mg., and 17-hydroxycorticosteroid excretion to 2.3 mg. per twenty-four hours; the levels of plasma 17-hydroxycorticosteroids fell to less than 3 μg. per 100 ml. for both the free and the glucuronide-conjugated fractions. The minimal degree of urinary mammotropic activity present before operation could not be demonstrated at this stage. The decline in thyroid function and urinary gonadotropin excretion and the impairment of adrenocortical reserve were, in conjunction with the significant clinical improvement, considered to be valid evidence of complete pituitary ablation.

After hypophysectomy—June 1957 (thyrotoxicosis)

Clinical history. In June 1957 the patient suddenly felt weak and ill, and according to her husband her condition deteriorated within a few days. She became nervous, had palpitations and a tremor, suffered from insomnia, lost her appetite and became nauseated. She was re-admitted to the hospital with a provisional diagnosis of recurrent breast carcinoma and liver metastases, complicated by an anxiety state.

No evidence was found indicating reactivation of the tumor, but she continued to be extremely restless and failed to improve after heavy sedation with meprobamate and barbiturates. She had continued to take 25 mg. of cortisone twice daily and thyroid 60 mg. twice daily and her weight had fallen from 126 pounds (58 Kg.) to 96 pounds (43.2 Kg.) within two months. She had a persistent sinus tachycardia of 120–140 per minute, her skin was soft and warm, and the coarse tremor of her fingers grew worse. Reluctantly a diagnosis of hyperthyroidism was considered. There were no abnormal eye signs and a thyroid adenoma was not palpated at this stage, but basal metabolic rates were +52 and +48 per cent. Oral thyroid therapy was stopped in preparation for further tests of thyroid function but her symptoms continued unchanged. Late in July 1957, six weeks after the onset of symptoms, a small adenoma about 1 cm. in diameter was felt in the right lobe of the thyroid.

Laboratory investigations (after July 1957). Results of all thyroid function tests confirmed the diagnosis of hyperthyroidism (Table 1). The BMR was consistently between +45 and +52 per cent even after thyroid had been discontinued for eight weeks, and the serum cholesterol concentration had fallen from a previous average value of 220 mg. to a level between 110 and 125 mg. per 100 ml. Results of radioactive iodine studies also indicated hyperthyroidism; plasma PBI^{131} activity was 2.3 per cent per liter and there was an extremely high conversion ratio of 96 per cent. Adrenal function, as judged by findings with the cortisone withdrawal test, continued to be severely impaired (Table 2). Urinary gonadotropin was still low, but again the extract was toxic. No significant quantities of urinary estrogens (estrone, estradiol-17β or estriol) could be found, and there was no mammotropic activity. Apart from hyperthyroidism, there was no evidence of target-organ function.

Subsequent treatment and progress

The symptoms and signs continued to be characteristic of severe hyperthyroidism and the clinical diagnosis of thyrotoxicosis was suggested independently by several

TABLE 1. SUMMARY OF THYROID FUNCTION DATA (CASE I)

Time	Clinical assessment	Serum cholesterol (mg./100 ml.)	BMR (%)	I^{131} studies				
				Thyroidal uptake	Urinary excretion	Total plasma activity	Protein bound I^{131}	Conversion ratio (%)
				(% dose at 24 hrs.)		(%/liter at 48 hrs.)		
Before hypophysectomy	Euthyroid	190	− 2	40	43	0.4	0.14	35
After hypophysectomy								
4 weeks	Euthyroid	270	−22	24	49	0.39	0.02	5
9 weeks	Euthyroid	280	—	12	70	0.12	0	0
48 weeks	Hyperthyroid	125	+50	—	—	—	—	—
56 weeks	Hyperthyroid	110	+45	75	7	2.4	2.3	96
2 years	Euthyroid	160	+15	—	—	—	—	—
Normal range		150–260	−20 to +10	15–45	40–70	0.2–0.9	0.07–0.2	15–45

physicians who did not know her history. During August 1957 a firm nodule in the right lobe of the thyroid increased in size to 3 cm. × 1.5 cm., while the rest of the gland parenchyma remained impalpable. Although the nodule took up I^{131} avidly, the remainder of the gland did not. Antithyroid treatment was begun with carbimazole in a dosage of 60 mg. a day. Improvement was slow, but after eight weeks the tremor was less obvious, and the BMR had fallen to +15 per cent. The thyroid nodule increased in size for about two months, but then became progressively smaller and by April 1958 could no longer be felt. As its activity became controlled the patient's general condition improved and she gained 14 pounds (6.4 Kg.) in weight in six months. By August 1958 there were no signs of overactivity of the thyroid. The BMR was +15 per cent but it was still necessary to give carbimazole, 5 mg. three times daily.

CASE II

Before hypophysectomy (September 1957)

Clinical history. Miss I.D. was 48 years old. Carcinoma of the right breast developed in April 1954. Treatment consisted of radical mastectomy and an irradiation-induced menopause. She remained well until July 1957, when she complained of rheumatic pains. In August she fell; a pathologic fracture of her left femur occurred because of osteolytic metastases. She was thin, weighed only 106 pounds (48 Kg.), and was in considerable

TABLE 2. SUMMARY OF LABORATORY INVESTIGATIONS (CASE I)

Time	Plasma 17-OCHS (μg./100 ml.)		Urinary steroids (mg./24 hrs.)		Urinary gonadotropin (m.u./24 hrs.)	Urinary mammotropic activity	Urinary estrogens (μg./24 hrs.)		
	Free	Glucuronide-conjugated	17-OS	17-OHCS			Estrone	Estradiol	Estriol
Before hypophysectomy	20.1	20.6	6.8	10.2	96	±	—	—	—
After hypophysectomy	*72-hour cortisone withdrawal test*								
4 weeks	2.4	0	1.4	2.3	<24	0	—	—	—
48 weeks	0	0	2.2	0	<24	0	0	0	0
60 weeks	—	—	1.2	2.9	<24	—	0	0	0
Normal range	6–18	6–25	4–10	5–15	96–394	?	0.8–7.1	0–3.9	0.6–8.6

TABLE 3. SUMMARY OF THYROID FUNCTION DATA (CASE II)

Time	Clinical assessment	Serum cholesterol (mg./100 ml.)	BMR (%)	I^{131} studies				
				Thyroidal uptake	Urinary excretion	Total plasma activity	Protein-bound I^{131}	Conversion ratio (%)
				(% dose at 24 hrs.)		(%/liter at 48 hrs.)		
Before hypophysectomy	Euthyroid	165	− 2	24	65	0.32	0.09	28
After hypophysectomy								
6 weeks	Euthyroid	180	−20	19	72	0.24	0.03	12
6 weeks; after TSH	Euthyroid	160	−11	65	28	0.45	0.3	64
20 weeks	Euthyroid	200	−12	14	68	0.25	0.03	12
20 weeks; after TSH	Euthyroid	—	—	22	64	0.35	0.15	43

pain. The right lobe of the thyroid was larger than the left, but no adenoma was detected. There were no clinical signs of thyroid dysfunction.

Laboratory investigations. Thyroid function appeared to be normal (Table 3). Plasma 17-hydroxycorticosteroid concentration and urinary 17-oxosteroid (17-OS) and 17-hydroxycorticosteroid (17-OHCS) excretions were within normal range. Urinary gonadotropin was present in significant amounts but no mammotropic activity could be detected (Table 4). The hemoglobin level was 12.7 Gm. per 100 ml., serum alkaline phosphatase 24 K.A. units per 100 ml., and serum calcium 11.2 mg. per 100 ml.

After hypophysectomy (after October 1957)

Clinical history. Surgical hypophysectomy was carried out in October 1957 and appeared to be complete. There was immediate relief of bone pain and improvement in her general condition, with an increase in appetite and weight. One month after operation an adenoma about 2×3 cm. in size was found in the right lobe of the thyroid, but the rest of the gland had decreased in volume. It was at first thought probable that generalized atrophy of the gland parenchyma had merely made the adenoma more readily palpable, but subsequent events disproved this. Over the next month there was a definite increase in size to about 3×5 cm., the nodule being smooth and firm in texture. There were no signs of myxedema even though thyroid was not administered; the skin remained soft and warm and the voice normal. During the 72-hour cortisone withdrawal test there were definite signs of glucocorticoid deficiency.

Laboratory investigations. Studies of thyroid function six weeks after operation showed a slight decline as compared with the preoperative findings, but the results were within the normal range (Table 3). The BMR had fallen to −20 per cent, but the thyroidal

TABLE 4. SUMMARY OF LABORATORY INVESTIGATIONS (CASE II)

Time	Plasma 17-OHCS (μg./100 ml.)		Urinary steroids (mg./24 hrs.)		Urinary gonadotropin (m.u./24 hrs.)	Urinary mammotropic activity	Urinary estrogens (μg./24 hrs.)		
	Free	Glucuronide conjugated	17-OS	17-OHCS			Estrone	Estradiol	Estriol
Before hypophysectomy	12.4	5.8	5.3	8.8	96	0	—	—	—
After hypophysectomy	*72-hour cortisone withdrawal test*								
5 weeks	0	0	—	—	—	0	—	—	—
20 weeks	0	—	1.3	1.2	<6	0	0	0	0

I^{131} uptake was 19 per cent (almost all of which was in the adenoma) and the TPA/PBI conversion ratio had fallen to 12 per cent. She was given two doses of 10 I.U. of TSH (Thyrotropar, Armour) intramuscularly, and a considerable increase in thyroid function resulted. Thyroidal I^{131} uptake was trebled to 65 per cent, plasma PBI^{131} activity rose to 0.3 per cent per liter, and the conversion ratio rose to 64 per cent. Most of the increase in uptake appeared to be accounted for by increased activity of the previously dormant parenchyma. These investigations were repeated twenty weeks after hypophysectomy, the results before giving TSH being similar to those obtained in November 1957, with the exception that the gland parenchyma had finally become inactive and only the adenoma showed a significant uptake of I^{131}. On this occasion, after two doses of 10 I.U. of TSH, there was a smaller increase in thyroid function than on the previous occasion, presumably because there had been time for the atrophic changes to become more pronounced. Nevertheless, there was still a definite response on the part of the atrophic parenchyma, with an increase in thyroidal uptake, PBI^{131} activity and conversion ratio, which rose from 12 to 43 per cent. There appeared to be a relatively greater response to TSH by the dormant parenchyma than by the adenoma. These findings appear to confirm that TSH was not being secreted by the pituitary lobe remnants. The decline in plasma 17-OHCS values after withdrawing cortisone for seventy-two hours and the decrease in urinary 17-OS and 17-OHCS excretion indicated adrenal insufficiency. No significant quantities of urinary estrogens were present in February 1958, and no urinary mammotropic or gonadotropic activity could be detected.

Subsequent progress.

One year after hypophysectomy this woman is active and well. The thyroid adenoma persists but she appears to be euthyroid. Her weight is 122 pounds (55 Kg.). She does not need to take thyroid tablets, but she requires cortisone, 25 mg. twice daily.

DISCUSSION

In neither of these 2 patients was there reason to suppose that hypophysectomy had been incomplete. Both showed definite sustained objective improvement in the mammary carcinomatous condition. In our experience, after surgical hypophysectomy combined with irradiation from unscreened Au^{198} seeds there is usually no more than 1–2 per cent of the anterior lobe parenchyma left in the fossa, a few cells being scattered round the capsule without hypothalamic connections. At autopsy we have found no evidence of reconstitution of the pituitary stalk or regrowth of the anterior lobe in 45 cases. It is unlikely that these remnants, or the assumption of activity by rudimentary pharyngeal pituitary tissue as suggested by Müller (11), can be of functional endocrinologic significance. Sheehan and Summers (12) showed that even in patients with advanced Simmonds' disease whose death was directly due to pituitary insufficiency, 5 per cent of the anterior lobe might be present at autopsy.

After hypophysectomy, thyroidal activity declines rapidly due to the absence of TSH. Thus assessment of thyroid function is a useful method for determination of the completeness of pituitary ablation, and it correlates well with results of other tests of pituitary function (4, 13). The persistence

of normal or increased thyroid function after complete hypophysectomy is therefore anomalous. Although plasma TSH levels were not assayed, in view of our experiences with 100 hypophysectomized patients we consider it justifiable to conclude that in these 2 women the thyroid adenomas functioned independently of TSH secretion. In 3 other patients regression of diffuse goiters has been observed within one to two months of operation. Indeed, hypophysectomy has been recommended by Albeaux-Fernet and his colleagues (14) and by McCullagh *et al.* (2) as a worthwhile procedure in the treatment of severe exophthalmos of thyroid-pituitary origin. Yet the paradox of hyperthyroidism occurring in patients with panhypopituitarism has recently been described. Fajans (1) studied a woman who became thyrotoxic eighteen years after the development of panhypopituitarism following a postpartum hemorrhage. In the patient of McCullagh *et al.* (2) hyperthyroidism persisted for six months after destruction of the pituitary by electrocautery, but eventually myxedema became apparent. Werner and Stewart (3) observed severe thyrotoxicosis in a woman who at autopsy was found to have a large chromophobe adenoma with only a rim of normal anterior lobe tissue (about 5 per cent). No thyrotropic cells could be demonstrated in the tumor, and the hyperplastic thyroid appeared to be autonomous. In none of these cases was a thyroid adenoma present, but in all 3 there was definite evidence of thyrotoxicosis and of hypopituitarism.

The physiologic action of TSH and the part it plays in the development of thyroid adenomas and thyrotoxicosis is still uncertain (15, 16). Werner *et al.* maintain that there is no evidence in favor of hypersecretion of TSH in Graves' disease (17, 18), whereas Rawson (19), Gilliland and Strudwick (20), and Adams and Purves (21) believe that plasma TSH levels are abnormally high. It is generally agreed that no TSH can be detected by existing methods of assay in patients with hypopituitarism (20, 22). Moreover, it is undecided whether TSH or (as suggested by Dobyns and Wilson (23)) a different exophthalmos-producing substance (EPS) of pituitary origin produces the exophthalmos often found in thyrotoxicosis of the Graves' type. On general principles it is not surprising that thyroid adenomas are autonomous compared with diffuse hyperplastic goiters, since benign adenomas in other endocrine glands are known to be independent of tropic hormones. The thyroid cells in an adenoma may well be of a different metabolic type than those in the rest of the parenchyma, and if the necessary enzymes be present, thyroid hormone may be elaborated in the absence of the TSH required by the normal cell. There may therefore be some justification for considering toxic adenoma (secondary thyrotoxicosis) as a separate entity from Graves' disease (primary thyrotoxicosis) as far as pathogenesis is concerned.

Whatever the ultimate explanation of thyroid autonomy, it now seems

established that under certain conditions thyroid adenomas and sometimes the whole parenchyma may elaborate thyroid hormones without the stimulus of anterior pituitary secretions. The fact that severe thyrotoxicosis can develop in the hypophysectomised human subject supports the contention that thyrotoxicosis is not necessarily a pituitary-induced disorder.

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ADDISON'S DISEASE IN TWO BROTHERS*

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ABSTRACT

Two young brothers with idiopathic Addison's disease are reported, together with a brief discussion of the occurrence of "familial" Addison's disease. In both boys the onset of the disease was at the age of 3–4 years.

UP TO 1952 approximately 100 cases of Addison's disease in children had been reported in the literature, according to Talbot (1).

A definite familial tendency for Addison's disease was doubted by Gutman in his statistical study published in 1930 (2). At this time most of the autopsy cases revealed a tuberculous etiology. In one of the earliest reports of the familial incidence of Addison's disease, tuberculosis was the etiologic factor (3). However, there have been several reports of familial Addison's disease caused by degeneration of the adrenal cortices of unknown etiology (4–11). In most of these instances, the diagnosis in at least one member of the family was made clinically without substantiation by adequate steroid studies or autopsy findings; therefore, an unequivocal claim for familial occurrence could not be made. More recently, 5 cases of familial Addison's disease, documented by steroid studies or autopsy findings, have been reported (12–16). Observations have also been made of the familial occurrence of adrenal insufficiency in infancy associated with adrenal hyperplasia but no virilism (17), and in identical twin infants with the adrenogenital syndrome (18). It has not been possible to evaluate some of the reports of familial Addison's disease cited by Berlin (13) and by Brøchner-Mortensen (8).

CASE REPORTS

Case 1

R.L. (9K534) was first admitted to the Peter Bent Brigham Hospital in April 1957, at the age of 10 years, for investigation of hyperpigmentation.

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The father was of Welsh and English origin with a dark complexion; the mother was of Irish, Scottish and English origin with a medium complexion. Both were in good health. The maternal grandmother was said to have been more "tanned" than usual. Two sisters and 4 brothers were well (Addison's disease subsequently developed in one of the brothers (Case 2)). The family history included cancer, hypertension and heart disease.

The past history included mumps, measles, chicken pox and (in 1954) a tonsillectomy.

The patient had always been of a fairly dark complexion and tended to tan more readily than other children. At the age of 4 years (1950), it was noted that he became more tanned than usual during the summer. In the three years prior to admission, generalized pigmentation became apparent and gradually increased; pigmentation of minor scars was first noted late in 1956. In the summer of 1955, recovery was slow from an attack of "flu" which included vomiting, diarrhea and crampy abdominal pains. Late in 1955, a bout of vomiting and diarrhea with a 19-pound weight loss and dehydration required intravenous infusions; recovery was slow. Up to this point he had had average or above-average ability in school and in sports, but after this illness there was a distinct change in the boy's personality. He became more easily fatigued, noisy, excitable, hyperkinetic and unable to concentrate. Enuresis was noted.

In March 1957, the patient was thought to have a urinary-tract infection for which he received sulfonamides. Later in the month, thyroid therapy was started and on the following day a fever and "red throat" developed. Several days later vomiting, crampy abdominal pain, and collapse with low blood pressure occurred. He responded to cortisone and intravenous saline, following which he was maintained on cortisone, 25 mg. orally per day, and desoxycorticosterone linguets, 2 mg. every other day.

Examination at this hospital in April 1957 revealed a generalized "dirty tan" discoloration of the skin, with pigmented scars and multiple dark spots, including one on the labial gingivae. One area of vitiligo was noted on the right chest posteriorly. He weighed 32 Kg., his height was 139 cm., and the blood pressure was 120/50 mm. Hg. Otherwise the findings on physical examination were not remarkable.

Laboratory findings were as follows: urine normal; stool, no occult blood; results of Hinton test negative; hematocrit reading 39.5 per cent; erythrocyte sedimentation rate, 10 mm. per hour; leukocyte count 7,500 cells per cu. mm., with a normal differential count; level of blood urea nitrogen, 14 mg. per 100 ml.; fasting blood sugar, 77 mg. per 100 ml.; serum sodium, chloride, potassium and calcium, 136, 103, 4.4 and 5.6 mEq. per liter respectively; serum phosphorus, 1.5 mMol. per liter; and carbon-dioxide combining power, 19.9 mMol. per liter. The 24-hour thyroidal I^{131} uptake was 33 per cent. An electroencephalogram and an electrocardiogram were normal as were roentgenograms of the chest, skull and abdomen. Bone age was 11 years. During a two-day control period the 24-hour outputs of urinary 17-ketosteroids were 4.6 and 3.7 mg., and of 17-hydroxycorticosteroids 1.3 and 1.9 mg. During a subsequent three-day period when a dose of 40 units of ACTH gel was administered intramuscularly twice daily (the patient was too uncooperative for ACTH intravenously) the 24-hour urinary outputs of total 17-ketosteroids were 2.9, 2.3 and 2.1 mg., and of 17-hydroxycorticosteroids 1.3, 2.1 and 1.5 mg. (virtually no response). A diagnosis of Addison's disease was made. At the time of discharge the patient was taking oral cortisone, 25 mg. daily, and 9-α-fluorohydrocortisone, 0.1 mg. daily. During the ensuing year the dosage of cortisone was increased to 37.5 mg. daily. The mother noted a decrease in pigmentation.

The patient was readmitted to the Peter Bent Brigham Hospital in February 1958. The weight was 38 Kg. (50–75th percentile), height 142 cm. (50th percentile), and blood

pressure 112/55 mm. Hg. The generalized pigmentation had decreased but otherwise his physical status was unchanged.

Pertinent laboratory findings included a negative tuberculin reaction using purified protein derivative in first and second strengths, and a negative reaction to an intracutaneous histoplasmin test. The 24-hour thyroidal I^{131} uptake was 33 per cent and the serum PBI level was 7.8 μg. per 100 ml. With a maintenance oral dose of 1.0 mg. of 9-α-fluorohydrocortisone daily, 24-hour urinary total 17-ketosteroid (17-KS) outputs were 2.7, 1.1, 2.2 and 1.5 mg., and 17-hydroxycorticosteroid (17-OHCS) outputs were 0.6, 0, 1.7 and 1.0 mg. on four successive days; on three subsequent days, during additional treatment with 40 units of ACTH intramuscularly twice daily (patient too uncooperative to allow intravenous ACTH), the comparable values were 1.9, 3.3 and 1.6 for 17-KS and 1.0, 2.7 and 1.3 mg. for 17-OHCS. An electroencephalogram and an electrocardiogram were normal, as were roentgenograms of the chest and skull. Thus, the previous diagnosis of Addison's disease was confirmed.

When the patient was discharged he was taking cortisone, 18.75 mg. daily, and 9-α-fluorohydrocortisone, 0.1 mg. daily.

Case 2

T.L. (3M206) was a brother of R.L. (Case 1). He was admitted to the Peter Bent Brigham Hospital in February 1958, at the age of 6 years, for investigation of hyperpigmentation of the skin.

The past history included measles, mumps and two attacks of tonsillitis within the prior two years, all tolerated well. A tonsillectomy in December 1957, was uncomplicated. A febrile illness with cough, rhinorrhea, abdominal pains and nausea (called "influenza") began about one month before admission and lasted between one and two weeks. Conditioned by the illness of the older brother, R.L., the parents had watched for pigmentary changes in the other children. They stated that the skin of T.L. had become darker in the previous three years, and especially so in the past year, with an associated blotchy appearance of the hands and face. The mother had also noted that T.L. tanned more easily than the other children and that there was less difference between exposed and unexposed areas in this boy compared to his siblings. His skin did not desquamate after exposure to hot sun, whereas the skin of the other children did. The mother also remarked that the scalp hair of both R.L. and T.L. was thinner than that of the other siblings. Generally the patient was well, was of a happy disposition and made friends easily. He had successfully completed kindergarten classes.

On examination, the boy weighed 21.4 Kg. (50–75th percentile) and was 115 cm. in height (50th percentile). The blood pressure was 104/58 mm. Hg. He was afebrile, generally healthy, active and cooperative. Pronunciation of words was noticeably imperfect. There was a diffuse tanning of the skin with a blotch appearance of the face and one area of vitiligo on the right chest anteriorly. Hyperpigmentation was present over the elbows, knees, joint lines and scars, and faint pigmentation on the alveolar mucosa exterior to the teeth. There was a mild generalized lymphadenopathy. Otherwise the physical examination revealed nothing unusual.

Laboratory findings were as follows: urine normal; hematocrit reading 39 per cent; leukocyte count 7,400 per cu. mm., with a normal differential count; erythrocyte sedimentation rate, 6 mm. per hour; level of blood urea nitrogen, 13 mg. per 100 ml.; fasting blood sugar, 77 mg. per 100 ml.; serum sodium and potassium, 139 and 4.2 mEq. per liter, respectively; serum phosphorus, 1.2 mMol. per liter; serum alkaline phosphatase, 3.6 Bodansky units per 100 ml.; no reaction to intracutaneous injection of histoplasmin,

nor to purified protein derivative (first and second strengths) in a tuberculin test. An electrocardiogram, and chest, abdominal and skull roentgenograms were normal. An electroencephalogram was considered borderline for the age of the patient and consistent with hypoadrenalism. During a three-day control period the 24-hour outputs of urinary total 17-ketosteroids (17-KS) were 2.8, 1.9 and 2.7 mg., and of 17-hydroxycorticosteroids (17-OHCS) 2.5, 0 and 0 mg. During a subsequent three-day period, when a dose of 24 units of ACTH was infused intravenously daily over eight hours, the 24-hour urinary outputs of total 17-KS were 1.0, 1.7 and 2.2 mg., and of 17-OHCS 0, 0 and 0 mg., indicating no responsive adrenocortical tissue. The plasma ACTH level was assayed twice (before steroid replacement therapy was started) by the method of Nelson and Hume (19); the values were 9 and 10 milliunits per 100 ml. of plasma. This was within the range previously found in this laboratory (8 ± 7 milliunits per 100 ml. of plasma) in Addisonian patients (20). A diagnosis of Addison's disease was made.

DISCUSSION

The 2 cases reported are interesting in several respects. First, both patients were children, and Addison's disease is relatively rare in this age group.

Second, in the absence of histologic proof to the contrary, it would appear that a degenerative process of unknown etiology accounted for the disease in these boys, since both were well during their early years of life. This conclusion is compatible with the observation of others that in children the usual etiology is not tuberculosis or an infiltrative condition, but an idiopathic atrophy or degeneration (1, 21, 22); and that in the very young child it may be related to one of a number of miscellaneous lesions such as cyst formation (23), congenital hypoplasia (24) or aplasia (25), or the presence of abnormal adrenal cells deficient in hormone production (26).

Third, T.L. (Case 2) on admission was clinically well and active (no medication), but urinary steroid excretion before and after ACTH therapy indicated little or no actively secreting adrenocortical tissue. Others (16, 27) have commented on the insidiousness and relative benignity of Addison's disease in children.

Fourth, the familial occurrence of this disease raises the possibility that genetic factors may play a role in idiopathic Addison's disease (6, 28), but to our knowledge there is no proof for such a view. Of interest however are: the repeated occurrence in more than one sibling; the occurrence of Addison's disease in more than one generation of a given family (8, 11, 12); and the association of Addison's disease with other defects presumed to be congenital, such as extra-lobed lungs (7), idiopathic spastic paraplegia (9) and multiple abnormalities (24, 26).

Fifth, the distinct temporal relationship of the initial Addisonian symptoms in several members of a given family closely associated geographically (14, 15) suggests the possibility that some common environmental influence may play a role.

The significance of the fact that in both of the patients presently reported the first sign of Addison's disease, *i.e.*, hyperpigmentation, appeared at the age of 3–4 years is not apparent.

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Letters to the Editor

INTRAVENOUS PROGESTERONE IN THE ANOVULATORY WOMAN

To the Editor:

The effect of progesterone on ovulation in the rabbit, heifer, rat and chicken has been well summarized by Cowie and Folley (1). In these species under controlled conditions, ovulation may be stimulated by the administration of progesterone. Our studies (2, 3, 4) of the metabolism of intravenously administered progesterone in humans and rabbits led naturally to an evaluation of the effect of this route of progesterone administration on human ovulation.

Seven patients received intravenous injections of 20 mg. of progesterone[1] for a total of 27 trials. These women were anovulatory, as indicated by amenorrhea and monophasic basal temperature curves. Progesterone withdrawal uterine bleeding (using vaginal progesterone[2]) had been previously demonstrated in each patient, suggesting the presence of an estrogenic endometrium.

Six patients who received 20 trials of intravenous progesterone responded with uterine bleeding on 17 occasions. The amount of bleeding was slight. It occurred five days after the injection of progesterone. There was no elevation of basal temperature. The seventh patient received intravenous progesterone on 7 occasions. In each trial, bleeding occurred as described. Moreover, on 6 occasions, at sixteen to eighteen days after the injection there was a rise in basal temperature which persisted for fourteen days. At the termination of the basal temperature elevation, uterine bleeding was observed. Biopsy at the time of bleeding showed a secretory endometrium. In control observations the patient continued to be anovulatory.

Thus endometrial bleeding was induced in the human subject with regularity by the intravenous injection of a small amount of progesterone. In one patient who was chronically anovulatory, evidence of ovulation was noted after progesterone withdrawal bleeding on 6 occasions.

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December 18, 1958

[1] Proluton, I. V. was generously supplied by Schering, A. G., Berlin.

[2] Colprosterone was generously supplied by Ayerst Laboratories, Inc., New York.

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THE EFFECT OF THYROTROPIN ON THE TURNOVER RATES OF THYROXINE AND TRIIODOTHYRONINE

TO THE EDITOR:

Recent studies (1–4) have shown an accelerated serum turnover rate of radio-*l*-thyroxine in hyperthyroid patients. In some instances, this acceleration persisted after the patient was made clinically euthyroid by therapy (1, 5). Since repeated injections of crude anterior pituitary extracts induce biochemical findings consistent with hyperthyroidism (6, 7), we decided to investigate whether injections of thyrotropin would accelerate the rate of removal of thyroxine or triiodothyronine from the circulation.

METHODS AND PATIENTS

Two athyroid patients, 1 patient with spontaneous myxedema, and 2 normal euthyroid subjects were studied. In the athyroid patients there was no demonstrable thyroidal activity, and in the case of primary myxedema the 24-hour thyroidal I^{131} uptake was less than 4 per cent. Both the athyroid and myxedematous patients were taking a daily maintenance dose of desiccated thyroid, and this was continued during the study.

To inhibit thyroidal uptake of the I^{131} contained in the administered radio-*l*-thyroxine, the euthyroid subjects were given 5 drops of saturated solution of potassium iodide three times daily.

Each of the patients was given 60–100 microcuries of *l*-thyroxine-I^{131} intravenously, and serial blood samples were drawn at intervals during the next eight to ten days. This schedule was repeated while a dose of 10 to 20 units of thyrotropin was given intramuscularly, either daily or every other day. Counts of radioactivity were made on 3-ml. aliquots of sera, using a Nuclear-Chicago scintillation well counter. The decline in total serum radioactivity was plotted on semilogarithmic paper.

RESULTS

In the 2 euthyroid subjects, the half-time of thyroxine was found to be in the normal range of 5.8 to 7.8 days (1), and the administration of thyrotropin caused no significant change.

Thyroxine half-times in the 3 athyroid or myxedematous patients were normal in 2 and slightly prolonged in the other. Their thyroxine half-times were not changed by thyrotropin (Fig. 1). Similar studies showed that thyrotropin did not alter the half-times of triiodothyronine-I^{131}. The results were evaluated using both the "F-test" and the "Rank-Order test" as described by Edwards (8).

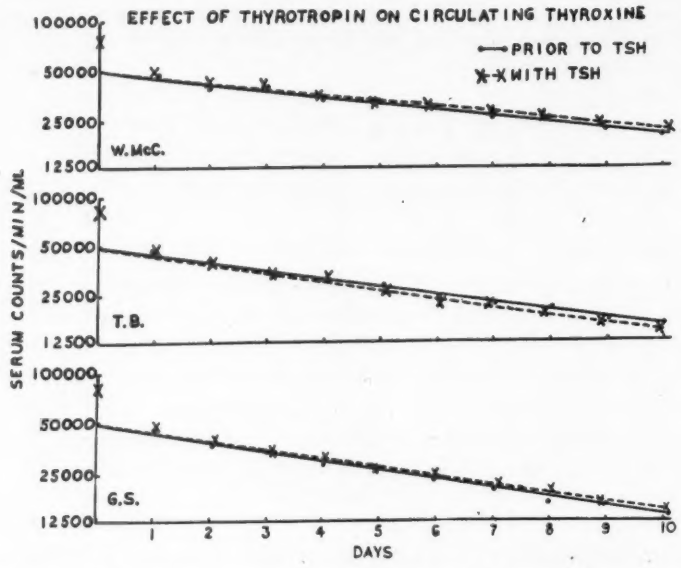

FIG. 1. Effect of thyrotropin on thyroxine half-time in 1 myxedematous patient (top graph) and 2 athyroid patients (lower graphs).

DISCUSSION

Although repeated injection of thyrotropin gives rise to other biochemical findings of hyperthyroidism, it does not induce the increase in turnover of thyroxine or triiodothyronine found in spontaneous hyperthyroidism. This indicates that the increased turnover in hyperthyroidism is secondary to an increased metabolic rate. Thus, thyrotropin does not have a direct "peripheral" effect on the rate of removal of thyroxine or triiodothyronine from the blood. The lack of effect of thyrotropin of the turnover rates of thyroxine and triiodothyronine in the normal subjects may have been due to the inhibitory effect of iodides. These findings do not rule out the

possibility that another form of thyrotropin, present in the serum of patients with Graves' disease (9), may have this effect.

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January 23, 1959

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The contents of this article are the personal views of the Air Force author and are not to be construed as a statement of official Air Force policy nor as Air Force endorsement of any commercial product described.

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The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

(For program, see the April issue of the Journal)

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman

in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

PROGRAM

Monday (A.M.)	**November 9, 1959**
8:00–9:00	Registration
9:00	Introductory remarks
9:15	*Hypothalamic and Pituitary Syndromes.* (Chairman: Dr. Lloyd.) Hypothalamic regulation of anterior pituitary function—Dr. Green. Chemistry of anterior pituitary hormones—Dr. Li. Case presentations—Panel discussion: Hypothalamic syndromes, hyperpituitarism, hypopituitarism, surgical and irradiation therapy—Drs. Adams, Escamilla, Green, Lawrence, Li and Lloyd.
Monday (P.M.)	
2:00	*Metabolic bone disease and parathyroid syndromes.* (Chairman: Dr. Keating.) Metabolism of bone—Dr. Copp. Case presentations—Hyperparathyroidism, osteomalacia, osteoporosis and other metabolic bone diseases—Drs. Copp, Gordan, Keating and Urist.
Tuesday (A.M.)	**November 10, 1959**
9:00	*The Thyroid.* (Chairman: Dr. Rawson.) Nervous control of thyroid function—Dr. Greer. Pathologic physiology of Graves' disease—Dr. Rawson. Case presentations—Panel discussion; questions and answers.
Tuesday (P.M.)	*The Thyroid.* (Chairman: Dr. Dobyns.)
2:00	The transport and metabolism of thyroid hormones—Dr. Rall. The function of thyroid tumors—Dr. Dobyns. Case presentations—Panel discussion; questions and answers. The

panel discussions will be carried on by Drs. Dobyns, Greer, Keating, Rall, Rawson, Starr, Turner and Van Wyk.

Wednesday (A.M.) **November 11, 1959**

9:00 *The Adrenal.* (Chairman: Dr. Tyler.)
CNS control of adrenal secretion—Dr. Ganong.
The pharmacophysiology of ACTH—Dr. Di Raimondo.
The regulation of hydrocortisone secretion—Dr. F. Tyler.
The physiology of aldosterone—Dr. Conn.
Adrenal insufficiency—Dr. Forsham.
Physiology of adrenal medulla and pheochromocytoma—Dr. Goldfein.
Adrenal surgery—Dr. Hinman.

Wednesday (P.M.) *The Adrenal.* (Chairman: Dr. Forsham.)

2:00 Aldosteronism—Dr. Conn.
The adrenogenital syndrome—Dr. Tyler.
Cushing's syndrome—Dr. Forsham.
Adrenal disease in children—Dr. Van Wyk.
Panel discussion; question and answers—Drs. Forsham, Conn, Hertz, F. Tyler and Van Wyk.

Thursday (A.M.) **November 12, 1959**

9:00 *The Testis.* (Chairman: Dr. McCullagh.)
Embryogenesis of the gonads; testicular physiology—Dr. Segal.
Panel discussion: hypogonadism, infertility, sexual precocity, and the use of androgen therapy—Drs. Heller, McCullagh, Segal and Turner.

Thursday (P.M.) *The Ovary.* (Chairman: Dr. Greenblatt.)

2:00 The physiology of reproduction; ovarian physiology and pathology; precocious puberty; the endocrinology of adolescence; use of newer progestational steroids in fertility and infertility; endocrine changes in pregnancy; and endocrine changes in the menopausal and postmenopausal periods—Drs. Fluhmann, Greenblatt, Lloyd, Page and Tyler.

Friday (A.M.) **November 13, 1959**

9:00 *Endocrine Aspects of Cancer Palliation.* (Chairman: Dr. Hertz.)
Panel discussion—Drs. Goldman, Gordan, Hertz, Pearson and Rawson.

Friday (P.M.) *Diabetes and Hypoglycemia.* (Chairman: Dr. Williams.)

2:00 Insulin metabolism—Dr. Williams.
Action of insulin on the liver—Dr. Chaikoff.
Action of insulin on muscle and adipose tissue—Dr. Renold.
Contrainsulin factors: insulin resistance—Dr. Renold.
Hypoglycemia: unstable diabetes—Dr. Williams.
Mechanism of action and clinical usage of oral drugs—Dr. Miller.
Management of diabetic complications—Dr. Forsham.
Lipotrophic diabetes—Dr. Miller.
Panel discussion; questions and answers—Drs. Chaikoff, Forsham, Miller, Renold and Williams.

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Announcement is made of a Special Traineeship Program under the direction of the National Institute of Neurological Diseases and Blindness, one of the seven institutes of the National Institutes of Health.

To qualify for an award, the candidate should have an M.D., Ph.D. or other equivalent degree, and at least three years of training or experience pertinent to the training for which he seeks support. The applicant must be an American citizen or have filed a Declaration of Intent. Traineeship awards generally are made for not less than nine months and not more than one year. However, all awards are subject to renewal for periods up to five years. Stipends are determined individually in accordance with each applicant's qualifications and the financial support needed to obtain the training applied for. Such stipends may range from $6,500 to $17,500 a year.

Special Traineeships frequently are utilized by basic scientists and clinicians to obtain prolonged and thorough training in highly specialized areas related to their previous experience. Other candidates seek to increase competence and knowledge in a particular facet of a chosen field during a shorter period of time.

Requests for complete information about the Special Traineeship Program and application forms should be addressed to Chief of Extramural Programs, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda 14, Maryland.

NEW YORK HEART ASSOCIATION, INC.

A two-day Scientific Session of the New York Heart Association will be held *December 11 and 12, 1959*, at the Hotel Biltmore in New York and will be devoted to the general subject "Salt and Water." Papers by twenty well-known investigators will be presented. A dinner meeting on December 11 will be addressed by Dr. George Wald of Harvard University on "Salt, Water and Vertebrate Metamorphosis." Accommodations are limited; For advance registration, write: The Medical Director, New York Heart Association, Inc., 10 Columbus Circle, New York 19, N. Y.

SYMPOSIUM ON RECENT DEVELOPMENTS IN RESEARCH METHODS AND INSTRUMENTATION

The Ninth Annual Instrument Symposium and Research Equipment Exhibit will be held *September 28 through October 1, 1959*, at the National Institutes of Health, Bethesda, Maryland.

Sponsors of the exhibit are the nation's leading instrument manufacturers, who will display the newest developments in laboratory glassware and electronic, surgical, radiation, optical, gas-sampling, and other research equipment.

Sponsors of the symposium are the Washington, D. C., sections of the American Association of Clinical Chemists, American Chemical Society, Instrument Society of America, Professional Group on Medical Electronics of the Institute of Radio Engineers, Society of American Bacteriologists, and the Society for Experimental Biology and Medicine.

For additional information about the symposium and exhibit, write to James B. Davis, National Institutes of Health, Public Health Service, Bethesda 14. Marvland.

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ANNUAL MEETING • ATLANTIC CITY, N.J. • JUNE 4, 5, & 6, 1959

Present status of "A.P.L." therapy in cryptorchidism

TREATMENT IS CONSIDERED AT LOWER AGE LEVELS
HIGHER DOSAGE REGIMENS ARE NOW RECOMMENDED

Many clinicians advocate therapy with chorionic gonadotropin between the ages of 5 to 7; others suggest instituting treatment at ages ranging from 3 to 14.[1-8] Those practitioners who advocate therapy in early childhood or before puberty do so in the belief that a delay in treatment may result in irreversible aspermatogenesis. In most cases, 10 to 12 years would appear to be an appropriate age to start therapy. In addition, larger doses of chorionic gonadotropin than those formerly employed are now recommended on the basis that these will produce a greater incidence of satisfactory responses.[3]

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Definitive use of "A.P.L."—A large percentage of cryptorchid testes will descend following therapy with "A.P.L."

Preoperative use of "A.P.L."—Therapy will usually facilitate orchiopexy increasing the size of the testes and the lengths of the cords.

Postoperative use of "A.P.L."—Follow-up therapy may be employed as an aid to preventing retraction of the testes.

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[1] 4,000 I.U., three times weekly, for two to three weeks, or
[2] 1,000 I.U., three times weekly, for six to eight weeks.

Caution: Evidence of sexual precociousness is an indication for the immediate withdrawal of treatment. After regression of undue development, reduced dosage regimen may be resumed.

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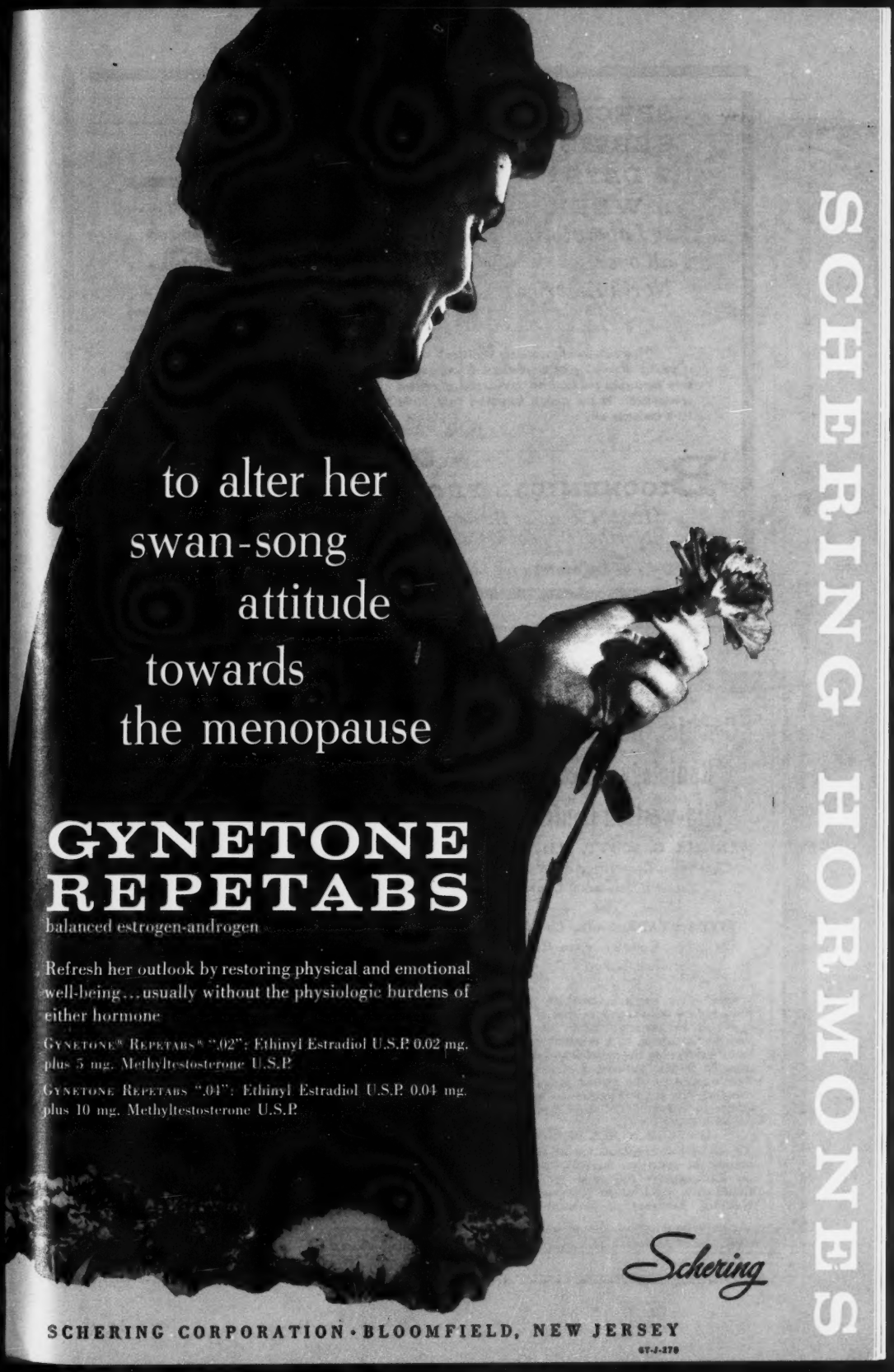
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Within these pages is complete coverage of probably **the most timely subject of this nuclear** age—how to keep all forms of controllable ionizing radiation to a minimum so that humanity can enjoy the benefits and developments afforded by the harnessing of nuclear energy. The interest of the public is focused on the problem—the medical profession has intensified efforts to minimize radiation dosage from the use of x-rays.

To aid in the dissemination of accurate information in radiation hazards, the Committee on Radiobiology requested the Mid-Western Conference, held under the auspices of the National Academy of Sciences-National Research Council. **Participants included 4 professors of genetics, 4 professors of radiobiology, and 21 professors of radiology.**

RESULTS: *To develop better understanding of problems arising from medical use of ionizing radiation among geneticists, radiobiologists, and radiologists*

PLAN: *Geneticists and radiobiologists could teach radiologists about genetic hazards and radiologists in turn could show what is being done to minimize radiation exposure*

PURPOSE: *Participants would disseminate the information through teaching programs to residents, interns, medical students and staff physicians*

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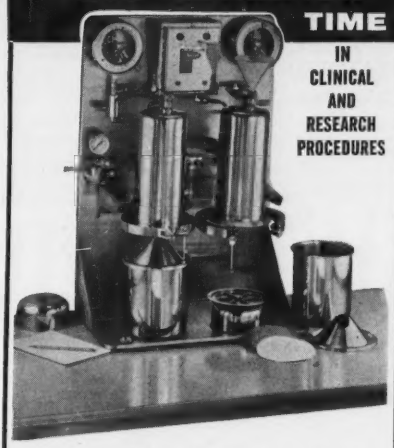

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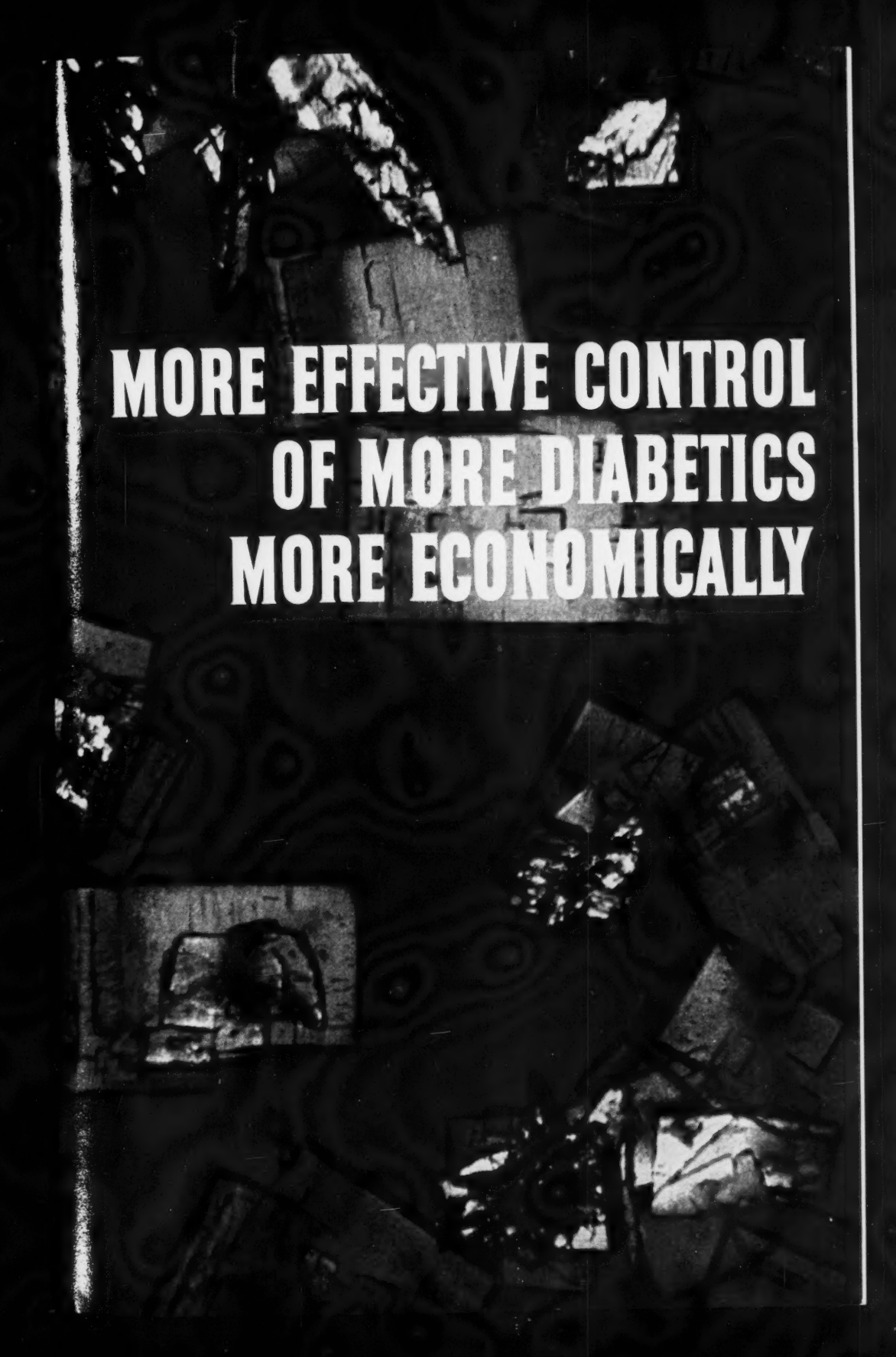
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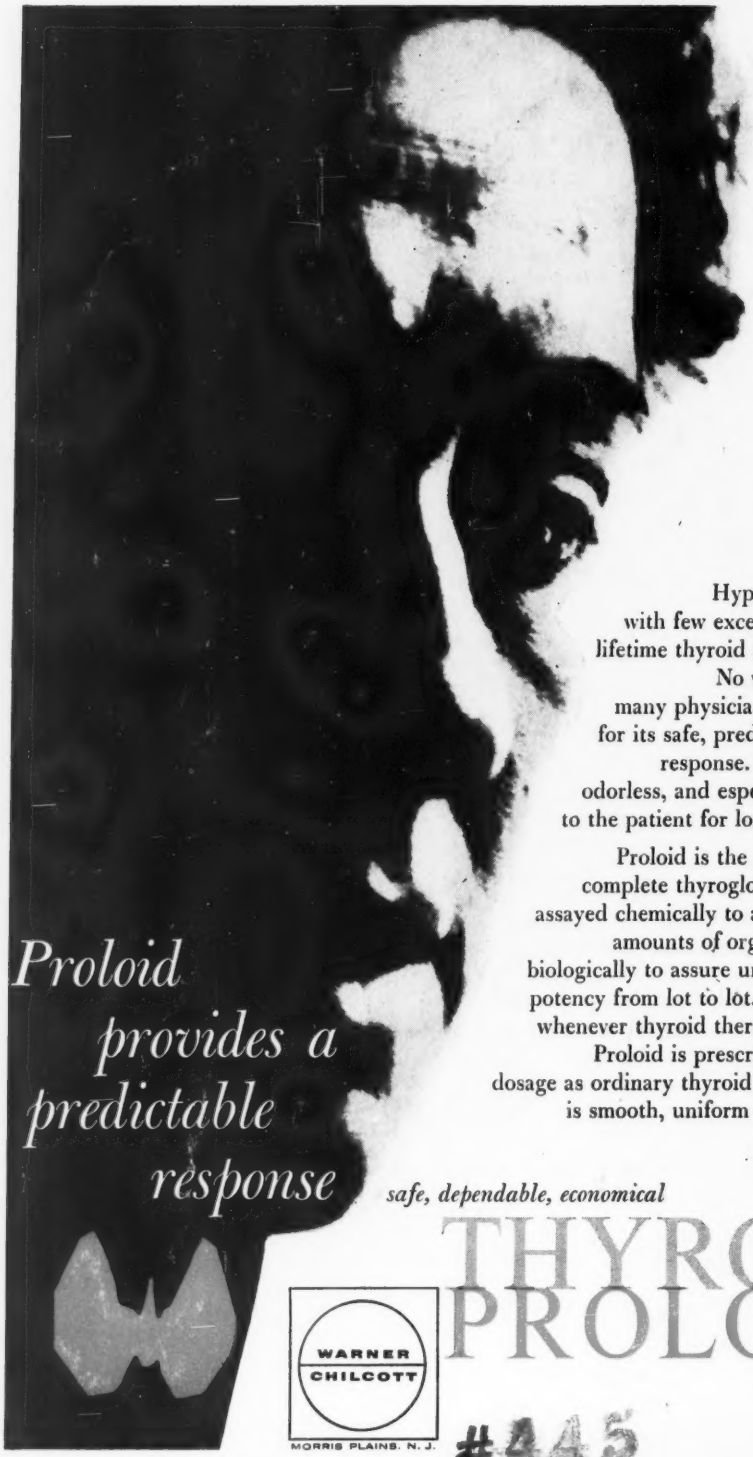

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